THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 80 MARCH 1, 1927 No. 1

THE INTERPRETATION OF THE INTRAVENTRICULAR PRESSURE CURVE ON THE BASIS OF RAPIDLY SUMMATED FRACTIONATE CONTRACTIONS

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Received for publication September 17, 1926

The intraventricular pressure curve of the mammalian heart is a graphic record of the tension developed by an after-loaded muscle. Its form, amplitude and duration are determined, therefore, in part by dynamic conditions which affect the load; in part also by inherent modifications of the contraction process itself. When the pressure curve is recorded by apparatus of adequate efficiency, it has a distinctive contour which has been described and analyzed by several investigators (deHeer, 1912; Piper, 1913–1914; C. Tigerstedt, 1913–1914; Garten, 1915, and Wiggers, 1921). Hitherto this has been done on the assumption that all fractions of muscle enter into the contractile state simultaneously or nearly so. The experiments of Erfmann (1913) indicating that all points on the ventricular surface are negative approximately at the same time, seemed to form an experimental foundation for such a view. These observations were not substantiated, however, by Lewis and Rothschild (1915), who found, on the contrary, that intrinsic negativity appears over different points of the ventricular surface at appreciably different times. If this be true the normal intraventricular pressure curve may not be regarded as an addition of ultimate contractions in phase but as a summation of many rapidly succeeding fractionate contractions.[1]

In view of the fact that the only existing observations as to the incidence of ventricular negativity are at variance, it seemed important to reinvestigate this question before attempting a further analysis of the intraventricular pressure curve.

METHODS. The negative potential developed at various points on

[1] The term "fractionate contraction" was introduced by the writer (1916) to designate the contraction of the smallest imaginary units of muscle tissue.

the ventricular surface was recorded in rapid succession and in conjunction with intraventricular and aortic pressure curves. The experimental procedure employed has recently been described in detail (Wiggers, 1925). Suffice it to recall that the unipolar method of leading was adopted. Six to eight cotton threads, soaked in saline, were stitched to the epicardium and tied to non-polarizable clay electrodes. These, in turn, were connected to a rotary switch which made it possible to connect each point in succession with the thread of a string galvanometer, the circuit in each case being completed through an indifferent electrode placed beneath the skin of the neck region. In this way, the incidence of negativity as related to the pressure curves could be recorded from eight different points in an interval, less than 2 minutes.

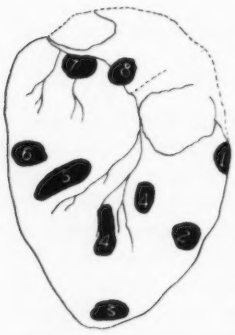

Fig. 1. Sketch, showing position and extent of areas within which punctate leads were taken in different experiments incorporated in table 1, and also giving system of enumerating leads.

After each experiment the heart with threads attached was excised and an outline sketch showing the exact location of threads was made. Figure 1, which shows a sketch of the areas in which threads were attached, also explains the system of enumeration employed in the leads. The interval between the appearance of negativity at any two points, which measures the difference in their excitation, is designated as the *interpunctal interval*. Thus, the interpunctal interval, *3–1* (fig. 1) gives the difference in excitation between left ventricular apex and base and similarly the interval, *5–8*, indicates the time difference in the excitation of the "central area" of the right ventricle and the pulmonary conus.

As the records of punctate negativity were obtained in succession some standard of comparison must obviously be employed. Two methods can be used:

Method I. The intervals between the earliest intrinsic deflections (taken at the moment of the first sharp upstroke) and the first elevation of intraventricular pressure during ventricular systole are determined and compared. When intrinsic negativity precedes the rise of pressure the figure is indexed with a plus sign; when it follows, by a minus sign. The algebraic difference gives the interpunctal interval. Specimen records in which this method was employed may be seen in an earlier communication (Wiggers, 1925).

Method II. The right auricle is artificially stimulated by rhythmic break induction shocks delivered at a rate slightly in excess of the natural. The instant of stimulation is directly recorded on the electrocardiogram as well as by a sensitive electric signal in circuit with the primary coil.

The interval between the moment of auricular stimulation and the appearance of intrinsic negativity over different points on the ventricular surface is measured. The difference between any two of these conduction intervals gives the interpunctal interval. On the whole, this method is the preferable one not only on account of the greater ease in making calculations but also because possible effects of heart rate changes are avoided. For typical records the reader is referred to the illustrations of the succeeding article in this issue.

RESULTS. I have recently (1925) published—but in support of quite another argument—data from 21 experiments showing the relation of intrinsic negativity over various surface areas to the rise of intraventricular pressure (method I). The results already presented show clearly that intrinsic negativity does not develop over the whole ventricular surface in constant relation to the elevation of intraventricular pressure. From these data the magnitude of the interpunctal intervals—which gives an index of the time required for the excitation of separate areas—can readily be determined. Such calculations show that the interpunctal intervals, 3–1 (left apex and left base) range from 0.003 to 0.023 second, averaging 0.013 second; the interpunctal intervals, 5–1 (central area of right ventricle and left base) show differences of 0.106 to 0.03 second, averaging 0.123 second.

In table 1, accompanying this paper, results from 21 other experiments are presented in which the conduction intervals from auricle to different points on the ventricular surface were determined by the second method. From these values the interpunctal intervals given in table I were calculated.

A perusal of this table shows that without exception the wave of negativity reaches the central area of the right ventricle first (area 5). Without exception, the apex of the left ventricle (area 3) is negative before the basal portions of the anterior ventricular surface (area 1) and as a rule, also, before the middle aspect of the left anterior surface (area 2). There is some variation in the latter, however. In one experiment (C354) the apex is negative slightly later than the middle of the left ventricle and in 2 experiments (C357, C362) these regions are negative practically simultaneously. On the whole, however, these data as well as those previously reported confirm the observations of Lewis and Rothschild (1915) as to the sequence in which the anterior surfaces of the heart are excited.

For our purpose, the real significance of this table lies in the magnitude of the interpunctal intervals. The greatest differences on the right and left ventricles respectively, as well as the greatest interpunctal intervals over the entire surface, are shown in the last 3 columns. A comparison of the interpunctal intervals, 3–2, shows that as a rule there is little difference in the incidence of surface negativity over the apical and middle

TABLE 1

Conduction intervals from right auricle to different areas on ventricular surface and interpunctal time-differences in intrinsic negativity

EXPERIMENT NUMBER	AREA 1	AREA 2	AREA 3	AREA 5	AREA 6	AREA 7	AREA 8	INTERPUNCTAL INTERVALS ON LEFT VENTRICLE	INTERPUNCTAL INTERVALS ON RIGHT VENTRICLE	GREATEST INTERPUNCTAL INTERVALS
C352 II	0.136	0.133	0.120	0.112		0.125		(3–1) 0.016	(5–7) 0.013	(5–1) 0.024
XIII	0.137	0.132	0.119	0.116		0.125		0.018	0.009	0.021
C353 II	0.160	0.153		0.147		0.157		(2–1) 0.007	(5–7) 0.010	(5–1) 0.013
XIII	0.160	0.153		0.148		0.155		0.007	0.007	0.012
C354 III		0.152	0.156	0.134		0.150		(3–2) 0.004	(5–7) 0.016	(5–2) 0.018
VIII		0.150	0.153	0.135		0.151		0.003	0.016	0.015
IX		0.150	0.152	0.132		0.150		0.002	0.018	0.018
C356 II	0.153	0.148	0.140	0.137		0.157		(3–1) 0.013	(5–7) 0.020	(5–1) 0.016
III	0.156	0.150	0.148	0.137		0.152		0.008	0.015	0.019
VII	0.166	0.162	0.158	0.145		0.162		0.008	0.017	0.021
C357 II		0.150	0.150	0.135		0.147		(3–2) 0.000	(5–7) 0.012	(5–2) 0.015
C358 IX		0.208	0.192	0.168		0.187		(3–2) 0.016	(5–7) 0.019	(5–2) 0.040
C361 IV		0.159	0.150	0.142		0.160		(3–2) 0.009	(5–7) 0.018	(5–2) 0.017
IX		0.155	0.149	0.139		0.152		0.006	0.013	0.016
C362 I		0.152	0.151	0.135			0.151	(3–2) 0.001	(5–8) 0.016	(5–2) 0.017
II		0.152	0.151	0.136			0.152	0.001	0.016	0.016
C363 I		0.173	0.165	0.155		0.170		(3–2) 0.008	(5–7) 0.015	(5–2) 0.018
C365 I		0.181	0.180	0.161		0.169		(3–2) 0.001	(5–7) 0.008	(5–2) 0.020
II		0.186	0.180	0.161		0.170		0.006	0.009	0.025
C372 I		0.160	0.150	0.137	0.151	0.153	0.160	(3–2) 0.010	(5–8) 0.023	(5–2) 0.023
C373 II		0.180	0.178	0.167		0.161	0.190	(3–2) 0.002	(5–8) 0.023	(5–2) 0.013
C374 I	0.189	0.185	0.180	0.174			0.192	(3–1) 0.009	(5–8) 0.018	(5–1) 0.015
C375 I	0.199		0.181	0.176	0.184		0.209	(3–1) 0.018	(5–8) 0.033	(5–1) 0.023
C376 II		0.161	0.155	0.138		0.160		(3–2) 0.006	(5–7) 0.022	(5–2) 0.023
C377 II	0.162		0.142		0.140	0.157	0.158	(3–1) 0.020	(6–8) 0.018	(6–1) 0.022
C378 I	0.182	0.172	0.159	0.142	0.149	0.160	0.170	(3–1) 0.023	(5–8) 0.028	(5–1) 0.040
C381 I	0.153	0.147	0.131	0.130	0.125	0.129	0.143	(3–1) 0.022	(6–8) 0.018	(6–1) 0.028
C382 II	0.158	0.152	0.139	0.138	0.140	0.148	0.158	(3–1) 0.019	(5–8) 0.020	(5–1) 0.020
C384 XVII	0.190		0.171	0.163			0.188	(3–1) 0.019	(5–8) 0.025	(5–1) 0.027
C387 VI	0.185		0.176	0.159		0.175	0.182	(3–1) 0.009	(5–8) 0.023	(5–1) 0.026

anterior portions of the left ventricle. A comparison of the interpunctal intervals, 3–1, shows however, that in 8 experiments the values ranged from 0.013 to 0.023 second, averaging 0.0185 second.

Similar differences were found on the right ventricular surface. In 3 experiments (C352, C353, C365) the interpunctal intervals, 5–7, ranged from 0.008 to 0.013 second, and in 7 experiments (C354, C356, C357, C358, C361, C363, C376) from 0.012 to 0.022 second, averaging 0.017 second. In 11 experiments where the interpunctal intervals, 5–8, or 6–8, were calculated they ranged from 0.016 to 0.033 second, averaging 0.018 second. In the last column of table 1 the greatest interpunctal intervals obtaining in the anterior surface of the heart are recorded. These data show that these interpunctal differences range from 0.012 to 0.04 second, averaging 0.021 second.

In this survey of the excitation of the ventricular surface, which for experimental reasons was limited to their anterior aspects, it was found that differences of about 0.02 second occur. Inasmuch as the interventricular septum is undoubtedly excited considerably in advance of the central area of the right ventricle and as the more basal regions on the posterior aspect of the left ventricle are probably excited still later, these values by no means express the extreme differences between the excitation of the first and last fractions of ventricular muscle.

These observations, therefore, confirm those of Lewis and Rothschild (1915), and supply corroborative evidence that the ventricular contraction in the mammalian heart must be regarded as a resultant of a rapid series of fractionate contractions which neither begins nor ends synchronously.

THE INTERPRETATION OF THE VENTRICULAR PRESSURE CURVE. In 1920 Koch presented a theoretical conception as to how summated fractionate contractions of the frog's ventricle enter into the composition of the contraction curve recorded by the suspension method. It is not difficult to adapt such a conception to the intraventricular pressure curve and we may attempt its analysis on a somewhat similar basis. To facilitate such an analysis let us assume 1, that normally an interval of 0.036 second elapses between the excitation of the first and last muscular fractions in the left ventricle; 2, that the isometric contractions of all units produce equal and similar effects on intraventricular pressure, and 3, that this effect can be expressed by a curve of isometric fractionate contraction, which is somewhat similar in form to that obtained from skeletal muscle (Fulton, 1925). The general contour of the fractionate contraction curve adopted is in harmony with the known facts of isometric contraction in skeletal muscle as determined by Fulton (1925) and accords also with approximately isometric contractions recorded by the writer from points

on the auricular surface. The curve chosen starts abruptly, continues as a straight line for 0.04 second, becomes convex to the abscissae, reaching its catenary in another 0.06 second and then continues as a straight line for another 0.02 second. It then relaxes abruptly in an arc concave to the base line (cf. curve a–a^1, fig. 2). The precise details of the assumed fractionate curve are, however, relatively unimportant; indeed, the facts which it is sought to establish hold equally well if we adopt a curve with an essentially different contour.

We may plot a series of such fractionate curves at equidistant intervals on coördinate paper and, by their algebraic summation, construct a resul-

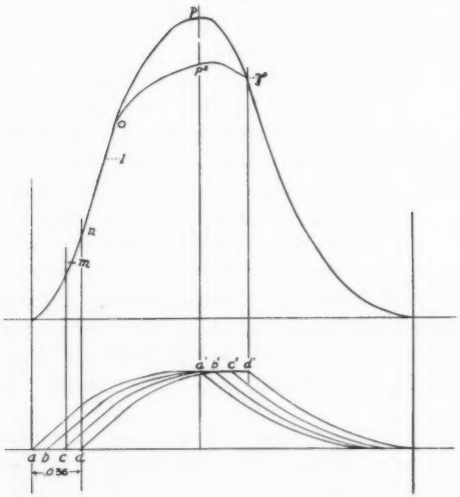

Fig. 2. Diagram showing how successive fractionate contractions (a-a', b-b', etc.) when summated produce an isometric intraventricular pressure curve, p, from which the after-loaded curve, p^2, is derived. Discussion in text.

tant pressure curve, keeping in mind that an innumerable number of similar curves must be thought of as interposed between them at exceedingly minute intervals.

Figure 2 shows curves accurately constructed on cross section paper in this manner but with coördinate lines eliminated for reproduction. Four such fractionate curves (a, b, c and d) are laid off so that their respective contractions and relaxations follow each other by 0.012 second; in other words, we assume that all fractions have been excited within 0.036 second. The points, a^1, b^1, c^1 and d^1, indicate the moments of their abrupt relaxation. Algebraic summation of these several fractionate curves during con-

traction and relaxation results in the isometric pressure curve, labeled *I*. On this curve, all fractions have entered a contractile phase at the point *n* and all fibers have begun to relax at *r*. Inasmuch as, in the body, the semilunar valves open and close at *o* and *r* respectively, the full pressure maximum is never reached, and the curve acquires a dome that is lower and more rounded, a form that may fairly be pictured as the curve—op^2r.

It is obvious that, by such algebraic summation of a series of fractionate contractions, having an entirely different contour, it is possible to construct an intraventricular pressure curve, which is not dissimilar to those actually obtained experimentally under good circulatory conditions (fig. 3).

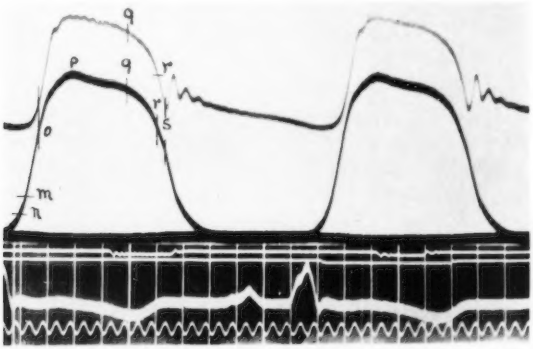

Fig. 3. Aortic (upper) and ventricular (middle) pressure curves together with an electrocardiogram (lower) recorded on rapidly moving paper. Tuning fork 0.02 second. Discussion in text. (½ actual size.) (N. B. Letters *m* and *n* should be reversed to accord with fig. 2 and text description.)

Such a construction supplies the key to the interpretation of certain details of the pressure curve which have hitherto remained imperfectly understood. The pressure curve begins with a concave elevation of pressure which often appears to become appreciably steeper at *m* but which does not reach its catenary until the point *n*. This early phase of gradually increasing tension has previously been interpreted as due to the closure of the A-V valves (Wiggers, 1921). This explanation does not hold, for the change is much too gradual and the inflection point persists almost unaltered when the A-V valves are rendered insufficient. Indeed, as I have shown in collaboration with Feil (1922) this portion of the isometric pressure curve is affected very little by valvular insufficiency. According to the theory of summated contractions, this concave phase of pressure

elevation is accounted for by the entrance of new fractionate contractions and the inflection to a straight line at *n* occurs as soon as all fractions participate in the contraction. This early phase of isometric contraction (fig. 3, up to *n*) may, therefore, be designated as the *entrant phase.*

If such an interpretation is correct we may expect a certain relationship between the greatest interpunctal differences on the surface and the duration of this entrant phase. I have estimated their relationship carefully and arranged the results of 11 experiments successively in table 2.

A glance at this table shows that, on the whole, the duration of the entrant phase increases as the interpunctal intervals become longer. The differences which range from 0.005 to 0.023 second and average 0.013 second are quite as constant as can be expected when we take into con-

TABLE 2

EXPERIMENT	INTERPUNCTAL INTERVALS, 5—1	ENTRANT PHASE	DIFFERENCE
C386 X	0.018	0.028	0.010
C382 II	0.020	0.031	0.011
C373 IV	0.020	0.037	0.017
C372 I	0.023	0.033	0.010
C381 I	0.023	0.036	0.013
C375 I	0.023	0.046	0.023
C387 VI	0.026	0.034	0.008
C384 XVII	0.027	0.032	0.005
C347 II	0.030	0.040	0.010
C348 II	0.030	0.051	0.021
C350 IV	0.044	0.054	0.010
Average difference			0.013

sideration the fact that the points from which intrinsic deflections were led, naturally varied somewhat in different dogs.

We may continue our analysis of the pressure curve, shown in figure 3. From *n* the pressure ascends as a straight line until the opening of the semilunar valves at *o*. With the rapid expulsion of blood, the ventricle contracts in an approximately isotonic or, more strictly, in an allassotonic manner. Since the gradient and contour of the ascending limb of the dome and, also, the actual moment when the pressure summit is reached are determined by the balance between cardiac and peripheral physical factors, little information as to the details of muscle activity can be obtained from this early portion of the dome. Katz and I (1922) have demonstrated by means of volume curves, however, that after the point of maximum pressure has passed, the velocity of systolic discharge decreases rapidly and progressively until, a short interval before the end of

systole, no blood is being expelled. In other words, during the descending portion of the dome (p-r) the ventricular contraction approaches an isometric character again, and gives a measure of evidence, at least, as to muscular activity in the ventricle.

This latter portion of the pressure dome, therefore, demands closer study and comparison with theoretical curves constructed as the resultant summation of fractionate curves. The sharp fall of pressure at r (cf. figs. 2 and 3), corresponding to the beginning of the incisura in the aortic pulse and responsible for the sudden closure of the semilunar valves, is our best index of the end of dynamic systole. Is it also the end of all contraction in the ventricle? While theoretical considerations indicate that it is, experimental facts do not warrant the conclusion that it is more than a reasonably close approximation of the time when all contraction ceases in the ventricle. A careful inspection of records taken on rapidly moving paper (e.g., fig. 3) shows that this is not the first pronounced bend in the curve toward the end of systole. In fact, after a point approximately placed at q, the declining limbs of *both* the aortic and ventricular pressure curves show evidence that tension energy is being subtracted in progressively increasing amounts. This progressive diminution of tension toward the end of systole may, of course, be interpreted as a characteristic of cardiac muscle when contracting under approximately isometric conditions; if so, we must assume that the contraction of cardiac muscle is different from the strictly isometric contraction of skeletal muscle recorded in an accurate manner (Fulton, 1925). On the other hand, these characteristic effects during late systole may clearly be explained by the fractionate offset of contractions. Thus, in the summated curve of isometric fractionate contractions shown in figure 2 as curve I, the summit p is reached when all fractions enter into the contraction process, but as fractions gradually pass into a state of relaxation, the gradient of fall becomes progressively steeper until the point r is reached. Such a gradual lapse of fractionate contractions probably also manifests itself in the latter part of an after-loaded contraction (p^2) especially as the ventricular contraction at this time approaches more and more nearly the isometric state.

This conception of a progressive and systematic deletion of fractionate contractions before ventricular systole actually ends is also supported by the fact that the T-wave of the electrocardiogram not only begins but even reaches its crest (or trough) long before the end of systole as marked by the incisura (fig. 3). Whatever explanation of the T-wave we choose to accept we always return to the fundamental assumption, more or less vaguely expressed, that it must ultimately be occasioned by the fact that some contractions cease before others. The interpretation of the intraventricular pressure curve on the basis of fractionate contractions

out of phase alone permits a concrete correlation of the electrical and dynamic changes arising in the ventricles toward the end systole.

The observation that the T-wave does not bear a constant relation to the end of systole (Garten, 1915) and that mechanical and electrical systoles do not vary consistently (Bartos and Burstein, 1924) does not invalidate the conclusion. The T-wave is a resultant effect produced by the progressive offset of contractions in the two ventricles which do not, however, proceed at the same rate and as Katz (1925) has shown, result in an asynchronous termination of mechanical systole on the two sides. Consequently, it appears to be entirely a matter of chance if the duration of the electrical systole of the whole heart corresponds to or varies proportionately with the mechanical systole calculated from a single ventricle. It follows from this analysis that the duration of dynamic systole, calculated from the beginning of the intraventricular pressure curve to the onset of the incisura, represents approximately the interval from the onset of the first to the end of the last fractionate contraction; it does not give the actual time that each fraction remains contracted. However, this fractionate contraction period may also be assessed by taking the interval from the inflection at n to the beginning of the incisura at r.

SUMMARY

The incidence of intrinsic negativity over different regions of the ventricular surface was recorded by unipolar leads and in conjunction with the pressure curves from the ventricles and aorta. The observation of Lewis and Rothschild (1915) that the anterior surface of the ventricles is excited at appreciably different times was confirmed. Roughly speaking, the interpunctal intervals between the base and apex are a little less than 0.02 second. As the interventricular septum and posterior basal aspects of the ventricles are undoubtedly excited, respectively earlier and later, than the anterior surfaces, these values do not express the extreme differences in the excitation of the first and last fractions of ventricular muscle. The intraventricular pressure curve may, therefore, not be regarded as an addition of ultimate contraction in phase but as a summation of rapidly succeeding fractionate contractions.

The intraventricular pressure curve is analyzed on this basis and it is suggested that the early concave rise of pressure is an index of the time required for all fractions to enter into the contracting state. Data are presented which show that, in general, the duration of this *phase of entrant contractions* varies as the *interpunctal intervals*. These give the differences in the development of intrinsic negativity on the anterior ventricular surface. Attention is directed to the fact that the incisura of the pressure curve remains our best criterion as to the termination of all fractionate

contractions but that previous to this many fractions have progressively ceased their contraction and entered into relaxation. This harmonized with the interpretation of the T-wave of the electrocardiogram which not only begins but reaches its crest or trough long before the end of dynamic systole is reached.

The duration of dynamic systole calculated by standard methods for either ventricle represents very closely the interval which elapses between the inception of the first fractionate contraction to the end of the last fractionate contraction in the ventricle. The fractionate contraction period (i.e., the interval that each unit of ventricular muscle remains in a contracted state) is approximated by the interval from the point of inflection to the incisura.

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ARE VENTRICULAR CONDUCTION CHANGES OF IMPORTANCE IN THE DYNAMICS OF VENTRICULAR CONTRACTION?

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Received for publication September 17, 1926

It has been shown that many experimental procedures—among them changes in venous return, alterations in aortic resistance and variations in heart rate—affect the vigor and duration of ventricular contraction as interpreted by intraventricular pressure curves (for references cf. Wiggers, 1922, a). I have been continually impressed by the fact that there is no definite relationship between gradient, amplitude and duration of contraction. Thus, an increased ventricular filling—resulting either from an augmented venous return or from a prolonged diastole—produces an increased intraventricular pressure maximum and a steeper isometric rise of pressure which is accompanied by a prolongation of systole. On the other hand, an increased aortic resistance, which likewise produces an increased pressure maximum, causes an abbreviation of systole.

It is generally assumed that such changes in the pressure curve offer evidence that somewhat corresponding effects occur in the ultimate units of ventricular muscle. This would be true if all fractions of the ventricular myocardium contracted in phase. This is not the case, however, in the mammalian ventricle, for Lewis and Rothschild (1915) have demonstrated, what it has recently been my privilege to confirm (1926), that the different sections of ventricular muscle are not excited simultaneously. Consequently, the intraventricular pressure curve may not be regarded as an addition of ultimate contracting units in phase but as a resultant summation of a series of successive fractionate contractions. Under these conditions it is conceivable that the gradient and duration of the contraction as given by the pressure curve might alter if the rate of excitation of the individual ventricular fractions occurs more or less rapidly than is normally the case. In the communication of Koch (1920) referred to in a preceding paper, he presents a theoretical discussion in which it is shown that a more rapid rate of ventricular excitation can cause a steeper gradient, a higher summit and a shorter duration of the suspension curve from a frog's ventricle without the form or duration of the ultimate fractionate contraction being affected, while a slower sequence of fractionate contractions

leads to opposite effects in the summated curve, similarly. It is easy to theorize that this may also be true in the case of the intraventricular pressure curve. Thus, in figure 1 B, a series of fractionate contraction curves are plotted 0.012 second apart and all fractions are supposed to have entered into the contraction process within 0.036 second. Algebraic summation yields the intraventricular pressure curve *I*, A. In figure 1, C, a series of fractionate contraction curves having the same form and duration are

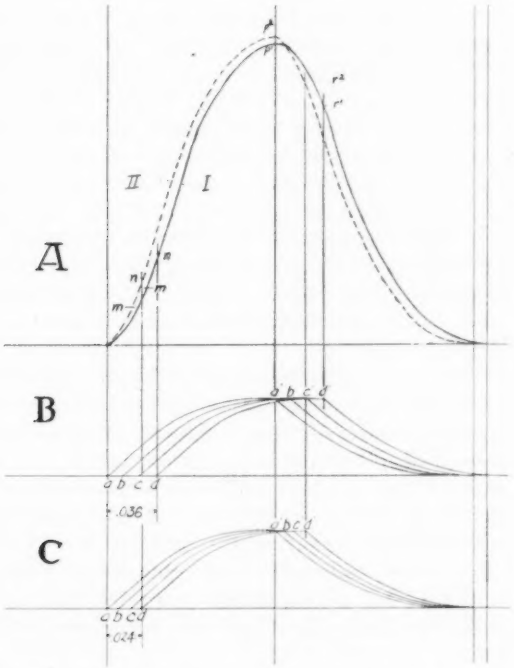

Fig. 1. Diagram showing the influence of different rates at which fractionate contractions are summated on the contour and duration of the isometric pressure curve of the ventricle (discussion in text).

similarly plotted but in such a way that they are 0.008 second apart, thereby reducing their time of entry to 0.024 second. Algebraic summation yields the isometric curve *II*, A. A comparison of curves *I* and *II* shows that a more rapid summation of fractionate contractions causes, *a*, earlier and lower inflection from a concave to a straight line, *b*, a steeper pressure gradient, *c*, an earlier termination of systole, and *d*, a more rapid rate of isometric relaxation. Opposite effects are produced when the entry of fractionate contractions is retarded.

There are experimental reasons for suspecting that such variations in the speed of fractionate summations may indeed affect the form and duration of the pressure curve, and may possibly account for the lack of relationship before mentioned. In 1925, I pointed out that the slower entry of fractionate excitation, which occurs when the ventricle is directly excited by an artificial stimulus, has a marked effect on the gradient, amplitude and duration of the pressure curve. In the preceding paper, I have called attention to the facts 1, that the differences in surface negativity as determined by the *interpunctal intervals* vary in different animals, and 2, that the length of these intervals corresponds quite well with the length of the entrant phase as determined by the point of inflection of the isometric pressure curve to a straight line (Wiggers, 1926). It remains for clear cut experimental work, however, to determine to what extent, if any, alterations of ventricular conduction and excitation *do occur* in the same heart when experimental conditions are altered and how far phasic change in the incidence of fractionate contractions, rather than fundamental variations in their form and duration, determine the dynamic consequences. This is indeed of fundamental importance for if the reactions of the heart as determined by experimental conditions are not solely referable to alterations in the character of the ultimate fractionate contractions many of our interpretations must be revised.

METHOD. The heart rate was kept constant by the application of rhythmic break induction shocks to the right auricle, except as noted in special cases. Left intraventricular and aortic pressure curves were recorded by optical manometers and the phases of the systolic period were calculated as in previous work (Wiggers, 1922) with the exception that the phase of entrant contraction as defined in the previous paper was added. Synchronously with these pressure curves, the incidence of intrinsic negativity over various regions was registered. The experimental method by which this was accomplished and the numerical system by which different areas of the ventricle are designated have been detailed in previous papers (Wiggers, 1925, 1926).

Figures 2 to 5 show specimen records. The moment of auricular stimulation was signaled both by the upstroke of a magnet and by a sharp deflection on the electrographic curve. The time interval between this recorded point of stimulation and the first sharp upstroke of the electrographic curve (intrinsic deflection) gives the time interval required for the impulse to pass from the auricle to a fixed point on the ventricular surface. Thus, in figure 2, it required 0.185 second for conduction of the impulse from the auricle to the basal anterior surface of the ventricle (area 1); 0.176 second to reach the apex of the left ventricle (area 3); 0.159 second to reach the central area of the right ventricle (area 5), etc. The differences between these values give the "interpunctal intervals" which serve

as an index of the speed of fractionate excitation. Such readings were uniformly made from 6 or 8 points of the ventricular surface, partly in the nature of checks, partly to make certain that no regions displaying variations in conduction were overlooked. Attention may be called to the fact that in the establishment of such conduction intervals it is desirable to measure 4 or 5 successive beats inasmuch as occasionally a much shorter interval occurs owing to an occasional escape of current to the ventricle. Such shortened intervals are indicated in the records of figures 2 to 7 by an asterisk (*).

THE EFFECTS OF INCREASED ARTERIAL RESISTANCE. Changes in arterial resistance are most readily induced by applying to the thoracic aorta a clamp that can be closed to a varying extent by means of a screw thread. Two or three series of optical pressure records and electrical variations were first obtained as controls, while the heart rate was kept constant by rhythmic break stimuli applied to the right auricle. The aorta was then compressed to a point where a distinct elevation of diastolic pressure occurred. In a few instances continuous records showing the immediate effects were taken. Specimen records are shown in figures 2 and 3, the former being a control and the latter showing the effects after partial aortic compression. In each case the calculated intervals for the phases of systole, entrant phase, isometric contraction, ejection, as well as total systole and cycle length, are written directly beneath the pressure curves. On the electrograms beneath the pressure records, the conduction intervals from the auricle to different areas of the ventricular surface are likewise given.

The effects on the pressure curves are characteristic and agree with those which have been previously described. The isometric contraction phase (a–c) is prolonged ($0.050 < 0.059$ sec.); but there is an abbreviation of systolic ejection ($0.155 > 0.136$ sec.) and of total systole ($0.250 > 0.195$ sec.) while the cycle remains unchanged. These effects may conceivably be due to an increased conduction rate and consequently a more rapid summation of fractionate contractions, or they may arise from specific changes in the fundamental fractionate contractions.

The pressure curve itself indicates, however, that phasic changes in the fractionate contraction are not concerned, for the duration of the phase of partial contraction (a–b) is not abbreviated and the inflection at b does not occur at a lower level as we should anticipate if the dynamic changes were due to an accelerated summation (cf. fig. 1).

This is also corroborated by a study of the conduction periods. A comparison of the conduction times in corresponding leads (*1*, *3*, *4*, *5*, etc.) shows that conduction from auricle to ventricle is slightly delayed, but in such a way that the interpunctal differences remain quite constant. Thus, the interpunctal interval, 3–1, changes from 0.009 to 0.007 second; the

interval, 3–4, is unchanged ; the interval, 5–7, changes from 0.016 to 0.017 second, while the interval, 5–8, increases from 0.023 to 0.025 second. The greatest differences are not more than 2 σ which may be considered within limits of error incurred in measurement.

Analyses for the total of 10 experiments made in a similar way are incorporated in table 1. In every instance aortic compression was accompanied by a steeper pressure rise, a prolonged isometric contraction, and with one exception, by a reduction in the phase of systolic ejection. As shown in the table there is rarely an abbreviation of the phase of entrant contractions (a–b), in fact, in more cases this appears to be slightly prolonged. Owing to the great difficulty in accurately determining the point, b, all of these variations are within limits of experimental error.

The results vary somewhat as regards the A-V conduction interval. In some cases this remains unaltered, in others it is slightly prolonged. In the latter case, however, the changes are quite equal in different areas of the heart, so that the interpunctal intervals are very slightly, or not at all, affected. The fact that the changes in either direction equal only a few σ and that typical dynamic changes occur within the ventricular pressure curve, is evidence that changes of intraventricular conduction play no essential part in determining changes in contour, amplitude or duration of the contractions as determined by the intraventricular pressure curves.

We must, therefore, conclude that the specific effects of an augmented arterial resistance on the ventricular pressure curve, especially the greater amplitude and shorter duration are due to a summation of augmented fractionate contractions with shortened contraction phases.

THE EFFECT OF INCREASED VENOUS RETURN. The influence of increasing the diastolic volume and initial length of ventricular muscle by increasing

Fig. 2. Normal aortic and intraventricular pressure curves with intrinsic electrical deflections from 6 points on the ventricular surfaces recorded in rapid succession and enumerated in accordance with diagram shown as figure 1 in preceding article. Actually each electrical record was accompanied by pressure curves such as are shown at the top but, as these were exact duplicates of the one shown, the electrical records were cut off and matched with the beginning of dynamic systole at points indicated by white arrows. Moment of auricular stimulation indicated by upstroke of signal record and by sharp deflections on curve (e.g., curve *I*). Numerals on curves indicate measured A-V conduction intervals to points corresponding to curve number. Numerals below pressure curves are in order: duration of entrant phase (a–b), of isometric contraction (a–c), of systolic ejection, of total systole and cycle length.

Fig. 3. Curves as in figure 3—during partial aortic compression. Discussion in text. Labels are as in figure 2.

Fig. 4. Curves as in figure 2—during saline infusion. Discussion in text. Labels same as in figure 2.

Fig. 5. Curves as in figure 2—marked dilated stage of infusion. Discussion in text. Labels as in figure 2.

Fig. 2

Fig. 3

Fig. 4

Fig. 5

TABLE 1

A-V Conduction intervals, interpunctal intervals and phases of ventricular systole. Effect of aortic compression

EXPERIMENT NUMBER	REMARKS	DIASTOLIC PRESSURE	AREA 1	AREA 2	AREA 3	AREA 4	AREA 5	AREA 7	AREA 8	INTERPUNCTAL INTERVALS ON LEFT VENTRICLE		INTERPUNCTAL INTERVALS ON RIGHT VENTRICLE		ESTRANT CONTRACT. PHASE	ISOMETRIC CONTRACT. PHASE	EJECTION PHASE	TOTAL SYSTOLE	CYCLE
C372 I	Control	45		0.160	0.150		0.137	0.153	0.169	(3–2)	0.010	(5–7)	0.023	0.033	0.067	0.111	0.144	0.320
II	Compression	79		0.162	0.151		0.146	0.158	0.170		0.011		0.024	0.037	0.089	0.089	0.177	0.318
C373 IV	Control	33		0.190	0.176		0.170	0.170	0.189			(5–8)	0.019	0.037	0.054	0.146	0.200	0.362
V	Compression	48			0.159		0.158		0.181				0.023	0.042	0.056	0.143	0.199	0.399
C375 I	Control	37	0.199		0.181		0.176	0.184*	0.209	(3–1)	0.018	(5–8)	0.033	0.046	0.061	0.181	0.242	0.431
II	Compression	67	0.201		0.187		0.174	0.185	0.207		0.014		0.033	0.037	0.070	0.168	0.238	0.430
C376 II	Control	49		0.161	0.155		0.138	0.169		(3–2)	0.006			0.029	0.058	0.115	0.173	0.346
IV	Compression	63		0.162	0.157		0.138				0.005			0.030	0.067	0.108	0.175	0.330
C377 II	Control	69	0.162	0.136	0.142		0.140*	0.157	0.158	(2–1)	0.026	(6–8)	0.018	0.022	0.042	0.172	0.212	0.458
IV	Compression	90	0.170	0.143			0.146	0.163	0.164		0.027		0.018	0.024	0.058	0.170	0.228	0.438
C381 I	Control	50	0.153	0.147	0.131		0.130	0.129	0.143	(3–1)	0.022	(5–8)	0.013	0.036	0.047	0.076	0.123	0.250
II	Compression	58	0.162	0.157	0.137		0.134	0.137	0.147		0.025		0.013	0.034	0.055	0.068	0.123	0.246
C382 II	Control	50	0.158	0.152	0.139		0.138	0.148	0.158	(3–1)	0.019	(5–8)	0.020	0.031	0.040	0.108	0.148	0.320
III	Compression	65	0.160	0.158	0.144		0.140	0.158	0.160		0.016		0.020	0.031	0.043	0.108	0.148	0.317

C384 XVII	Control	38	0.190		0.171		0.163		0.188	(3–1)	0.019	(5–8)	0.025	0.032			0.135	0.278
XVIII	Compression	39	0.191		0.171		0.164		0.190		0.020		0.026	0.034			0.131	0.278
C386 X	Control	38		0.150	0.154	0.150	0.142	0.142	0.162	(4–3)	0.004	(5–8)	0.020	0.028	0.053	0.146	0.199	0.357
XI	Compression	79		0.155	0.159	0.150	0.143	0.145	0.164		0.009		0.020	0.028	0.066	0.110	0.176	0.357
C387 VI	Control	60	0.185		0.176	0.197	0.159	0.175	0.182	(3–4)	0.021	(5–8)	0.023	0.034	0.050	0.155	0.205	0.398
VII	Compression	79	0.188		0.181	0.202	0.160	0.177	0.185		0.021		0.025	0.035	0.059	0.136	0.195	0.396

* Readings from "area 6."

the venous return was tested, as in the past, by infusing a solution of normal saline at body temperature. In these experiments the heart rate was also kept constant by artificial stimulation of the auricle by means of break induction shocks. As previously pointed out (Wiggers and Katz, 1922; DeGraff and Sands, 1925) a gradual infusion often causes no change, or at most, only a slight elevation of diastolic pressure, owing to the fact that changes in aortic resistance are usually avoided by the coincident reduction of viscosity.

In these experiments it seemed desirable, however, to give the infusion somewhat more rapidly, with the result that diastolic pressure occasionally rose slightly. To obviate this, the thoracic aorta was slightly compressed by a screw clamp previous to the infusion, and, after infusion, was slightly loosened again until the diastolic pressure was restored to normal.

Typical effects may be illustrated by continuing to use segments of the experiment from which those of figures 2 and 3 were taken. As, in this instance, infusion followed partial aortic compression, the curves of figure 3 may be regarded as a control.

The series of records in figure 4 illustrates the effects during the early stages of infusion. The pressure curves show typical changes described on previous occasions: a more pronounced auricular wave, *p*, an elevation of initial pressure, *a*, and a steeper rise of isometric pressure, *b–c*, the curve as a whole reaching a higher pressure maximum. The pulse pressure as shown by the amplitude of the aortic pressure curve is also larger. Calculations show that the phase of isometric contraction, *a–c*, is slightly reduced (from 0.059 to 0.052 sec.). The duration of systolic ejection and total systole are both increased ($0.155 < 0.159$) ($0.205 < 0.211$) in spite of a slightly reduced interval of diastole.[1]

The series of records of figure 5 shows the results when infusion had been carried to the point where the heart had become extremely dilated and the initial tension had decreased. All the effects, already alluded to, increase with the exception of the duration of total systole, which slightly declines, owing to the fact that the artificial stimulator had gradually gained speed and the duration of cycles also progressively decreased. The entrant phase, *a–b*, at first increases slightly but is subsequently reduced even below normal values ($0.039 > 0.032$). The fact that the inflection at *b* appears to be somewhat higher up on the curve indicates that this slight abbreviation is at least not entirely due to a more rapid summation of contractions. The conduction intervals shown in figures 3, 4 and 5, before and during various stages of infusion, show very minor changes. Thus, the conduction intervals marked on curves *I*, *III* and *IV* from the left heart are so nearly constant that certainly no interpunctal differences of

[1] Due to slight variation of the motor which actuated the stimulator.

importance exist. In the case of the right ventricle, which was dilated more than the left, the conduction intervals in curves *V*, *VII* and *VIII* all increase, but to an almost proportionate degree, leaving the interpunctal differences the same.

Similar observations were made in experiments on six different dogs in which the effects of infusion were studied from the incipient to over-distended stages. The tabularized data of table 2, show that as a rule the entrant phase is reduced in spite of the fact that the point of inflection, *b*, occurs at a higher level. This may lead to the logical conclusion that the summation of intraventricular fractions is accelerated. A study of the interpunctal intervals does not substantiate this, however, in fact such differences as occur would tend to operate in the opposite direction. Thus, as shown in table 2, the conduction intervals from auricle to ventricle increased somewhat at all points, but in one experiment (C347) they decreased slightly. Calculations listed in columns labeled "interpunctal intervals," show, however, that the time differences on the ventricular surface remain surprisingly constant, the greatest change noted being an increase of 0.004 second in the case of the left ventricular surface, and of 0.008 second in the case of the right ventricle. On the whole, however, the changes are much smaller or entirely absent. These results show that the duration of the entrant phase as well as its gradient depends not only on the rate of summation but on the character of the contraction process itself. The steeper the gradient of the fractionate contractions, the earlier and the higher the inflection point appears.

These results are significant, however, not only in eliminating the possibility that differences in the rate of impulsive propagation are concerned in determining the nature of the contractions, but also show that stretching of the myocardium has no effect on the rate of intraventricular muscle conduction. These results, therefore, support the interpretations of Schellong (1924) who found that the act of lengthening itself fails to alter ventricular conduction rate unless the accompanying changes in irritability increase the speed of excitation. If these observations may be correlated they show that stretching of the muscle in the mammalian heart produces its characteristic reactions by affecting the contractile process rather than the state of irritability.

EFFECT OF HEART RATE CHANGES. Inasmuch as the ability of cardiac tissue to conduct appears to be so intimately associated with the preceding recuperative interval, it appears probable that a progressive diminution of the recovery interval resulting from a progressive increase in the rate of excitation would have a tendency to prolong the conduction intervals. While this is true in a broad sense, it appears that different portions of the heart vary considerably as to the recovery period required for normal conduction. Thus, Lewis, Feil and Stroud (1920) found that the rate of

TABLE 2

A-V Conduction intervals, interpunctal intervals and phases of ventricular systole. Effects of increased venous return

EXPERIMENT NUMBER	REMARKS	AREA 1	AREA 2	AREA 3	AREA 4	AREA 5	AREA 7	AREA 8	INTERPUNCTAL INTERVALS ON LEFT VENTRICLE		INTERPUNCTAL INTERVALS ON RIGHT VENTRICLE		ESTRANT CONTRACT. PHASE	ISOMETRIC CONTRACT. PHASE	EJECTION PHASE	TOTAL SYSTOLE	CYCLE
C347 II	Control	0.191	0.186	0.176	0.183	0.161	0.166		(3–1)	0.015	(5–7)	0.005	0.040			0.218	0.392
III	Infusion	0.186	0.183	0.171	0.176	0.168	0.170			0.015		0.002	0.035			0.221	0.388
C348 II	Control	0.206	0.190	0.185	0.190	0.176	0.191	0.205	(3–1)	0.021	(5–8)	0.029	0.051			0.199	0.405
III	Infusion	0.217	0.200	0.196	0.199	0.180	0.200	0.210		0.021	(5–7)	0.020	0.040			0.222	0.409
											(5–8)	0.030					
C350 IV	Control	0.198	0.180	0.176	0.168	0.154	0.170	0.171	(3–1)	0.022	(5–8)	0.017	0.054			0.241	0.460
V	Infusion	0.202	0.182	0.178	0.174	0.158	0.171	0.178		0.024		0.020	0.047			0.241	0.459
VI	Infusion	0.200	0.180	0.178	0.167	0.160	0.170	0.177		0.022		0.017	0.043			0.233	0.455
C384 XXI	Control	0.180		0.165				0.187	(3–1)	0.015			0.031	0.051	0.088	0.139	0.280
XXIII	Infusion	0.180		0.165				0.190		0.015			0.029	0.049	0.113	0.162	0.281
XXIV	Extreme infusion	0.191		0.175				0.192		0.016			0.031	0.059	0.109	0.168	0.289
XXV	Partial recovery	0.197		0.178				0.198		0.019			0.029	0.059	0.100	0.159	0.282
C386 X	Control		0.150	0.154	0.150	0.142	0.142	0.162	(4–2)	0.000	(5–8)	0.020					
XIV	Control		0.150	0.152	0.148	0.138	0.138	0.160		0.002		0.022	0.034	0.050	0.132	0.189	0.339
XVII	Infusion		0.162	0.172	0.164	0.152	0.160	0.180		0.002		0.028	0.032	0.042	0.152	0.196	0.339
XVIII	Partial recovery		0.154	0.170	0.160	0.151	0.155	0.178		0.006		0.027	0.035	0.045	0.140	0.185	0.340

C387 VII	Control partial aortic compression	0.188	0.185	0.181	0.202	0.160	0.177	0.185	(3–4) 0.021	(5–8) 0.025	0.040	0.060	0.156	0.206	0.372
VIII	Infusion	0.190	0.187	0.181	0.204	0.165	0.183	0.192	0.023	0.027					
IX	Infusion, partial decompression	0.200	0.189	0.182	0.208	0.163	0.184	0.191	0.026	0.028	0.033	0.050	0.156	0.206	0.372
X	Partial recovery	0.190	0.190	0.180	0.203	0.163	0.182	0.186	0.023	0.023	0.038	0.051	0.151	0.202	0.360
XI	Further infusion	0.190	0.190	0.180	0.200	0.164	0.182	0.190	0.020	0.026	0.038	0.050	0.150	0.200	0.360

auricular conduction remains constant for rates up to 300 or 350 per minute, after which conduction rates increase somewhat abruptly; on the other hand, Lewis and Masters (1925) report an experiment which shows a gradual decrease in A-V conduction, even when the rate does not exceed 200 per minute, and this decrease progresses especially rapidly when the rate exceeds 250 per minute. Finally, Lewis and Rothschild (1925) had found by using the interpunctal differences on the surface of the heart that there was at most a "trifling" difference when the ventricle was stimulated directly at rates ranging from 170 to 300 per minute. Inasmuch as in these experiments the excitation spread in an unnatural manner over the ventricle, it appeared desirable to make a few experiments in which conduction occurred over natural pathways. Accordingly the conduction

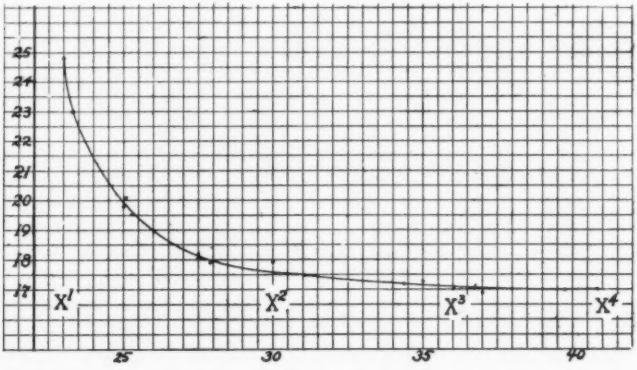

Fig. 6. Plot showing relation of cycle length A-V conduction. Discussion in text.

times from auricle to various ventricular points were established, when the rate of artificial stimulation was changed. From the figures so obtained values for the interpunctal intervals were calculated. Three experiments yielded consistent results of which one may be analyzed in detail. In experiment C387, the artificial rate was increased from one just above that normal for the animal to about 260, at which time a half-rhythm developed. The conduction intervals from auricle to point 3 (left apex) were calculated and plotted as shown in figure 6. The abscissae represent cycle lengths and the ordinates conduction intervals. It will be seen that these intervals increase very slightly at cycle lengths ranging from 0.40 to 0.30 second, after which they increase rapidly until cycle lengths of 0.23 second are reached. The plotted curves resemble those of Lewis and Masters (1925) on A-V conduction. At four points of this series (marked X^1 to X^4)

intrinsic negativity observations were made over all points of the heart, and the curves obtained at X^1 and X^3 are shown in figures 7 and

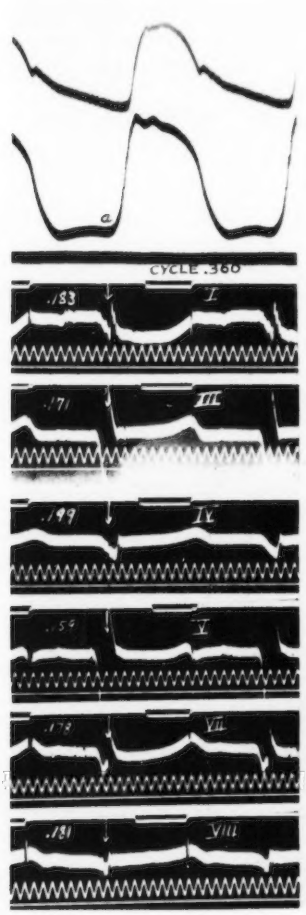

Fig. 7

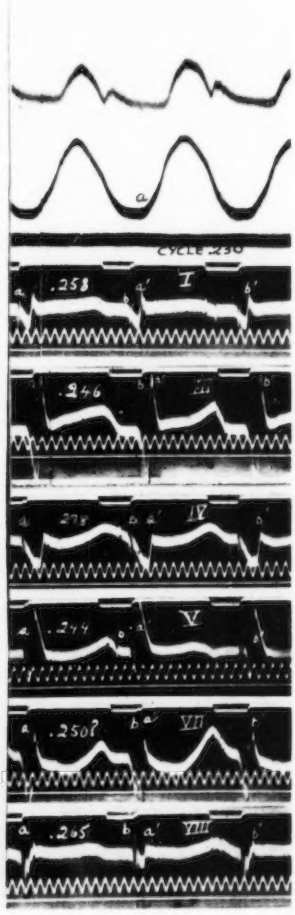

Fig. 8

Figs. 7–8. Curves as in figure 2 showing influence of heart rate on conduction time and constancy of interpunctal intervals. Labels as in figure 2. Discussion in text.

these, as well as in other experiments, the interpunctal intervals remained surprisingly constant, and that in spite of a marked prolongation of the conduction times. A comparison of figures 7 and 8 shows that in the left

ventricle the interpunctal interval (3–1) remains absolutely constant, 3–4 increases from 0.028 to 0.032 second; in the right ventricle the interpunctals (5–7) read 0.019 and 0.006 second,[2] respectively, while the interval 5–8 alters from 0.022 to 0.021 second. It is obvious that while the rate of excitation affects that A-V conduction to an appreciable degree it has very little influence on the conduction within the ventricular myocardium, at least up to a heart rate of 250 per minute.

There is no evidence, therefore, that the alterations in the gradient, amplitude, and duration of the pressure curves which accompany changes in the heart rate are produced as a result of variations in the rate of fractionate summation, but must be interpreted as specific changes in the ultimate fractionate contractions themselves.

The pressure curves obtained under these conditions are shown above the intrinsic negativity curves in figures 7 and 8. They illustrate the well known effects of rapidly increasing the heart rate by supraventricular stimulation. The gradient of the isometric pressure rise is slower, the amplitude is less and the phase of contraction is shortened considerably as the heart rate increases. During the entrant phase, *a–b*, the gradient is also less, its inflection at *b* is reached at a higher level and after a much longer time. Without the experimental observations that the interpunctal intervals remain practically unchanged this could undoubtedly be interpreted as indicating changes in the rate of fractionate summation. It is obvious that during changes in heart rate as well as during changes in the diastolic length, brought about by infusions, the duration of this phase is not solely determined by the rate of fractionate contractions but by their gradient as well.

Discussion. Physiologically, changes in venous return, arterial resistance, and heart rate have pronounced and characteristic effects on the gradient, contour, amplitude, and duration of the ventricular contraction process, as shown by intraventricular pressure curves. The results reported in this paper indicate that these effects may not be attributed to alterations of intraventricular conduction of such a nature that the individual fractions enter into the contraction process at times differing from normal. On the contrary, it appears that time of excitations is kept remarkedly constant under conditions that may be supposed to operate normally and even so, when A-V conduction intervals alter considerably. In establishing this, the essential purpose of this research is accomplished.

An important corollary follows, however, viz., that the intraventricular pressure curves may continue to be regarded as a criterion of changes in

[2] This marked reduction is dubious and accounted for by the difficulty in determining the points in segment VII accurately, owing to the fact that at these rapid rates the deflection due to the stimulus so clearly coincides with the initial deflection of the previous beat.

contraction which individual muscle units of the ventricle undergo, but only as long as the ventricles are excited over natural pathways from a

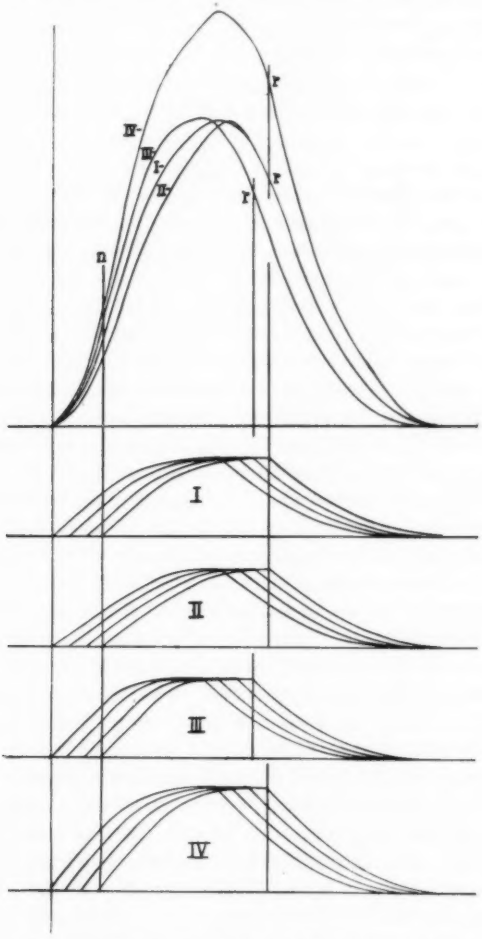

Fig. 9. Series of fractionate contractions and their summation effects as a pressure curve. *I*, control; *II*, summation of curves with more gradual gradient; *III*, summation of curves with steeper gradient and shorter duration; *IV*, summation of curves with steeper gradient and higher maximum.

supraventricular focus. This conclusion holds despite the fact that the intraventricular pressure curve represents a rapid serial summation of the

individual fractionate contraction; and also, despite the fact that the pressure curve developed by each individual fraction probably differs considerably both as to duration and contour from that recorded.

These conclusions can be clarified by a set of schematic curves shown in figure 9, which probably set forth after a fashion how these effects are brought about. Curve *I*, of the series of isometric intraventricular pressure curves (upper set) results from the algebraic summation of fractionate contraction curves drawn just below and also labeled *I*. The intraventricular pressure curve *II* (upper set) is derived by algebraic summation of fractionate contraction curves (labeled *II* below) which have a more gradual development of the pressure gradient. Similarly the intraventricular pressure curve *III* results from algebraic summation of the fractionate contraction curves (labeled *III* below) which have a steeper gradient of pressure development. While the gradient and duration of these fractionate curves vary they all reach the same pressure level. We note that the gradient of contraction affects the height of the inflection point, *n*, and the steepness of the isometric curve but does not alter the pressure maximum nor the duration of systole.

In curves *I* and *II* it is shown that as long as the separate fractions are excited at reasonably constant intervals the duration of the fractionate contractions is the sole factor which determines the duration of systole, as this event is measured by our criterion on the pressure curves. If, as was designedly done in the fractionate curves of series III, the duration of the individual fractionate contraction is abbreviated, the duration of total systole as determined by the pressure curves is also reduced (cf. points *r*).

Comparison of curves *I*, *II* and *III* also shows that differences in the velocity of pressure development per se can have no influence on the height of the intraventricular pressure curve. The maximum pressure in all cases equals the sum of the pressures during the catenary part of the fractionate contraction curves. The only way in which such an increased height can be brought about is through the development of a higher tension by each contracting fraction, or—what amounts to the same—by the possible introduction of previously inactive fractions into the total contraction process. This is illustrated by algebraic summation of the greater fractionate contractions of series IV which results in the intraventricular pressure curve also labeled *IV*, in the upper series of figure 8. Inasmuch as the development of a higher fractionate pressure level in the same interval of time is necessarily accompanied by an increased velocity of pressure development the gradient of the intraventricular pressure rise also becomes steeper.

The results of this research lead us to the following inferences: 1. The level at which the true inflection of the pressure rise to a straight line be-

gins, and the slant of isometric gradient appear to be determined by the velocity of pressure development in the separate fractionate curves and—as far as experiments go—by this alone. 2. The pressure maximum reached is entirely determined by the ultimate tension reached in each fraction and is not determined by the velocity of the fractionate contraction process. 3. The duration of the contraction curve is solely regulated by the duration of the fractionate contraction interval.

SUMMARY

There were a priori reasons for believing that variations in the speed with which the fractions of ventricular muscle are excited and summated *may* affect the gradient, height and duration of the resulting pressure curve. Experiments were carried out to determine to what extent alterations in ventricular conduction *do* occur when experimental conditions are altered and how far phasic changes in the fractionate contractions rather than fundamental variations in their form determine the dynamic consequences.

Results showed that during circulatory changes produced by increasing the aortic resistance (graded aortic compression), by augmenting the venous return and cardiac filling (saline infusion) and by altering the heart rate do not affect the "interpunctal intervals" of surface negativity even when considerable differences in A-V conductivity occur. Consequently, the general conclusion is reached that changes in ventricular excitation leading to alterations in the phasic summation of fractionate contractions play no rôle in producing the characteristic effects observed in the intraventricular pressure curves under these experimental conditions.

Changes in gradient, amplitude and duration of ventricular contraction evidenced by the intraventricular pressure curve may, therefore, continue to be regarded as evidence of similar changes in the character of the ultimate contractions as long as the ventricles are excited over normal pathways from a supraventricular focus. This conclusion holds despite the fact that the intraventricular pressure curve represents a rapid serial summation of individual contractions and despite the fact also that the pressure curve developed by each fraction of ventricular muscle probably differs constantly both as to character and contour from that recorded as the intraventricular pressure curve.

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AN INVESTIGATION OF THE EFFECT OF LONG-CONTINUED INGESTION OF ZINC, IN THE FORM OF ZINC OXIDE, BY CATS AND DOGS, TOGETHER WITH OBSERVATIONS UPON THE EXCRETION AND THE STORAGE OF ZINC

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Received for publication September 17, 1926

During the last two years, there has been in progress, in the Departments of Physiology and of Ventilation and Illumination of the Harvard School of Public Health, an extensive series of experiments designed to amplify the knowledge already available upon the widespread normal occurrence of zinc in plant and animal tissues and in animal secretions and excretions; to throw new light upon the normal zinc metabolism and upon the significance of zinc to the living organism; and, finally, to obtain information more precise and accurate than now exists in the literature upon the absorption, storage, and excretion of zinc, and upon the physiological and pathological action of zinc and its compounds upon the animal body.

The purpose of the particular experiments reported in this paper was to determine whether or not zinc, administered to animals (cats and dogs) by the gastro-intestinal tract and in the form of a non-corrosive zinc compound (zinc oxide) in fairly large doses, for long periods of time, would occasion acute or chronic damage of any sort, especially to the gastro-intestinal tract itself, to the kidney or to organs in which it might be stored; whether or not zinc taken in this way would be stored and, if so, in what organs; and by what avenues and in what amounts it would be excreted.

REVIEW OF THE LITERATURE. *Zinc absorption.* Zinc compounds taken into the gastro-intestinal tract are, according to Schlossberger (1848), Michaelis (1851), Sacher (1893), Dragendorff (1895), Von Jaksch (1910) and Bernatzik (quoted by Seiffert, 1918), converted in the stomach by the free hydrochloric acid or by free lactic acid, if it be present, into zinc chloride and zinc lactate. Both compounds are, according to the weight of authority, decomposed by the protein substances present, and protein compounds are formed which are either soluble or are in part dissolved by the free acids present. Rosow (1862), investigating the behavior of ingested zinc albuminates, introduced into the stomach of a fasting dog a bag filled with wet zinc albuminate. At the end of three hours, one-half of the albuminate was dissolved and at the end of twelve hours the bag was

empty. This indicates that zinc albuminates ingested or formed in the stomach are dissolved there, but the literature throws no light upon their further digestion, nor upon their chemical form when absorbed, nor their place of absorption. Large amounts of the more inert compounds such as zinc oxide may be only partially changed in the stomach or bowel, and considerable quantities probably pass out in the feces without any changes having occurred (Schlossberger). Indeed, Heller (1847) believed that zinc oxide was physiologically quite inert, that it passed through the gastro-intestinal tract unaffected, and that he could recover it quantitatively in the feces.

Cushny (1924), discussing the general question of the absorption of the heavy metals, wrote: "The form in which the metals are absorbed is quite unknown, but it is not unlikely that they are taken up in insoluble forms by the leucocytes and thus carried into the tissues." This statement is especially interesting to us in view of the fact that the white cell counts in our zinc-fed animals have tended always to be high, though never above the upper limits reported in the literature as occurring in normal animals.

Zinc transportation. The opinion appears to be general that zinc, once it is absorbed, is carried by the blood stream (Michaelis, Delezenne (1919), etc.). Whether the blood plasma or the blood corpuscles act as vehicles remains somewhat uncertain. The only data available on this subject are contained in a paper by Delezenne in which he reported experiments showing that the plasma contained approximately one-tenth only of the total zinc in blood, the rest being present in the cells. Red cells showed only a trace of zinc while leucocytes were very rich in it. This finding ought undoubtedly to be confirmed with the present more exact methods of analysis, especially since the figures given by Delezenne for zinc in mammalian blood, 15 to 25 mgm. per thousand, are decidedly higher than the figures which Lutz (1926) and Fairhall (1926b) in this laboratory have repeatedly obtained for rat, cat and human blood.

The question as to whether or not the lymph stream also transports zinc is another subject for further study. Michaelis investigated the zinc content of the thoracic duct of four horses which had been fed zinc oxide at various times before their death (one and a half to three hours before). In no case was he able to detect zinc in the thoracic duct lymph, although he found zinc in the blood of the portal vein of one of these animals which had twice, previously, taken 22.5 gm. of the preparation. The methods of zinc analysis current in Michaelis' day were not, however, delicate enough to detect very small amounts of the metal.

Zinc storage. Much has been written upon the question of zinc storage, following zinc administration, but, unfortunately, the great majority of investigators (Michaelis, Mazkewitz (quoted by Sacher), Lehmann (1897), Brandl and Scherpe (1899), Jacobi (1899), Seiffert, and Salant, Rieger and

Treuthardt (1918), etc.) report the finding of zinc in various organs and tissues, apparently without knowing that all organs and tissues have a normal zinc content which, once known, obviously becomes a matter of considerable importance before storage of zinc is inferred.

Owing to the absence of normal standards of zinc occurrence in tissues and to the frequent use of poor analytical methods as well, one is justified, in our opinion, in concluding from the literature no more in regard to the storage of zinc in the body in excess of the normal requirements than that the metal is taken up from the blood stream in large part by the liver and is temporarily stored in this organ during the process of gradual excretion. It is possible, but not proved, that zinc may also be stored in smaller amounts in other organs—e.g., the pancreas, thyroid, spleen, bone and kidney—but whether this storage is a transient one or whether some one organ or tissue may store zinc for years as the bone does lead (Minot, 1924) previous work on zinc gives us no convincing evidence.

Zinc excretion. The consensus of opinion (Schlossberger, Michaelis, Mazkewitz (quoted by Sacher), Sacher, Lehmann) in the literature in regard to the excretion of zinc is that absorbed zinc, taken up from the blood and temporarily stored by the liver, is excreted in small amounts by the liver into the bile, but, in the main, is gradually liberated by the liver to the blood stream and is then excreted in minor amounts into the urine by the kidney, but chiefly by the gastro-intestinal tract into the feces. According to Lehmann, the excretion of zinc through the cecum and colon is three to four times as great as through the stomach. Delezenne has reported the finding of zinc in normal pancreatic juice, an interesting fact in view of experimental results which we shall present.

Lewald (quoted by Sacher) brings some evidence to bear that, following the ingestion of zinc oxide, zinc is excreted in milk, but since he presents no figures for normal zinc in milk (Birckner, 1919) his observations are suggestive rather than conclusive. A high zinc content in the skin of animals to which zinc has been administered has been interpreted by Salant, Rieger and Treuthardt as indicating skin excretion of zinc. Recently, Drinker and Fairhall[1] have definitely proved the skin to be a route of normal zinc excretion by demonstrating the presence of zinc in normal sweat.

Studies on the excretion of zinc administered experimentally began as early as 1846 but the early work (Bouchardart, 1846 (quoted by Schlossberger), Heller, Schlossberger, Michaelis, Mazkewitz (quoted by Sacher), Sacher) has little or no significance for us because of the poor methods of zinc analysis used and because of the absence of figures upon normal zinc excretion.

Lehmann, studying the hygienic significance of zinc, fed 1052 mgm.

[1] Unpublished data. Personal communication.

of zinc carbonate (553 mgm. zinc) to a dog in a single dose and recovered in the urine, 8.5 mgm.; in the feces, 540 mgm.—a total recovery of 548.5 mgm. of zinc. In a similar experiment, the same author after the same dose of zinc, recovered 6.9 mgm. in the dog's urine and 400 mgm. in the feces (some lost). A third dog received zinc carbonate by mouth over a period of eleven months. During a period when the daily dose of zinc was 553 mgm., the daily output of zinc in the urine was 4 to 6 mgm. Here again, the normal zinc content of urine and of feces is not taken into account, nor have we any proof that all the zinc in the feces had been absorbed and excreted, though doubtless zinc carbonate is somewhat more soluble in gastric juice than zinc oxide, and, therefore, more likely to be completely absorbed. In spite of these objections, Lehmann's experiments offer qualitative proof at least that the major part of ingested zinc leaves the body in the feces, a minor part in the urine. From the high zinc content of the liver and the bile of his experimental animals, Lehmann inferred zinc excretion in bile, while high zinc figures for the cecum and colon (three to four times as high as in the stomach) led him to regard the large bowel as an important agent in ridding the body of this metal.

Brandl and Scherpe, studying the excretion and storage of zinc, fed zinc malate pills and a solution of the double salt of sodium zinc tartrate to rabbits, guinea pigs and dogs. The amount of zinc recovered in the feces was compared with the amount fed and was shown to constitute a very large fraction of the dose. For example, of 995 mgm. of zinc, given to a dog in the form of a solution of zinc malate mixed with meat, 967 mgm. were recovered in the feces and in the contents of the bowel at autopsy; again, of 420 mgm. of zinc (fed to a dog as the double salt of sodium zinc tartrate) 419 mgm. were recovered in the feces and in the intestinal contents. Although these recovery figures are not quite so good as they appear since the normal zinc content of food and of feces was not taken into account, nevertheless they do indicate, as does the rest of our qualitative evidence, that ingested zinc can be largely recovered in the feces. What proportion of it is absorbed and subsequently excreted, and what proportion passes through the bowel unabsorbed, we are not prepared to venture an opinion.

Following the feeding of zinc oxide to a calf and to a sheep, Lander and Edwards (1912) express the opinion that by far the greater part of the zinc ingested passes out in the feces as zinc sulphide.

Salant, Rieger and Treuthardt administered to animals by mouth, subcutaneously and intravenously zinc malate and zinc acetate. The fate of the zinc when injected intravenously indicated that the gastro-intestinal canal was the chief organ of elimination, since, by the end of two or three days after its administration, one-third to nearly one-half of the zinc given was recovered from feces and from gastro-intestinal contents. The urine, according to these authors, contained but minute quantities of

zinc, amounts which were practically within the limits of experimental error. Although the amounts of the metal present in the skin were small, the evidence, in the opinion of these investigators, was sufficient to warrant the conclusion that zinc may either be stored or eliminated by this organ.

Summarizing briefly the opinions expressed in the literature in regard to the excretion of zinc, we may say that absorbed zinc begins to be excreted promptly after its absorption. A small fraction leaves the body in the urine; the major amount, however, is excreted along the alimentary tract, some of it in the bile, possibly some of it in the pancreatic juice. If the absorbed zinc is greater in amount than the excretory mechanism can handle at once, zinc is temporarily stored, chiefly in the liver, and liberated by this organ as fast as it can conveniently be excreted. A small amount of zinc is excreted in sweat and probably some also in milk.

Local and general action of ingested zinc oxide. The literature contains a vast amount of data upon the results of feeding zinc oxide to man and to animals. In evaluating these data, there is, in our opinion, one all-important question which must be asked in regard to each group of experiments —Was the zinc oxide used chemically pure? This question is, in fact, applicable to all experiments with zinc oxide, regardless of the portal of entry, but we shall consider here only ingestion experiments. The chief contaminations to which zinc oxide is subject are lead, cadmium, arsenic and antimony—substances carried over from the ore in the process of manufacture. Occasionally investigators, working upon the effect of zinc and its compounds upon health, admit lead contamination (Murgia, 1906; Seiffert), and attempt to determine what part zinc plays in causing a given set of symptoms by excluding the disturbances obviously caused by lead, and attributing everything else untoward to zinc. Other writers (Landouzy and Maumené, 1850; Bouvier, 1850; D'Amore, Falcone and Maramaldi, 1892) describe rapid and overwhelming intoxications which they attribute to zinc oxide—intoxications so extraordinarily severe and so obviously and totally out of accord with the bulk of experimental evidence, that we are led inevitably to the conclusion that some contaminating substance, probably lead or arsenic, and not zinc oxide itself, occasioned the damage reported.

Experiments reported by D'Amore, Falcone and Maramaldi serve as an excellent illustration of the point which we wish to make. These investigators fed zinc oxide, "the purity of which had been well ascertained," to dogs. The dose at first was 1 gram daily; after three to five days, ½ gram daily. For the first four or five days the animals vomited one-half to one hour after eating; after this the oxide was retained. The dogs at once began to lose weight; they suffered from muscular weakness, which increased to such an extent that they were unable to move. Sensibility

disappeared first in the hind limbs. A remarkable pallor of the mucous membranes developed.

There was change in size and shape of the red cells, a diminution in their number, a tremendous increase in the number of white cells, and a great decrease in the amount of hemoglobin. The urine diminished in amount, and showed amounts of hemoglobin which increased steadily until it became a deep red in color. No red blood cells, however, were present. There was albuminuria (7 to 9 grams per liter of urine), glycosuria (a maximum of 15 to 17 grams of sugar per liter), and the urinary sediment showed hyaline and granular casts.

The dogs died on the tenth to the fifteenth day from the beginning of the experiments. Pathological examination of the organs obtained at autopsy showed, according to the authors, areas of inflammation in the stomach and duodenum (but not of a severity in accord with the importance of the intoxication), together with fatty degenerations of the liver, the pancreas, and the kidney, especially of the epithelium of the tubules. These findings should be borne in mind in order that they may be contrasted with the negative results which we obtained when, using zinc oxide of a high degree of chemical purity, we attempted to repeat these experiments.

With the possibility of contamination firmly in mind, let us now consider the conflicting mass of experiments which have been reported upon the ingestion of zinc oxide by men and by animals. One group of investigators (Heller, Wibmer (quoted by Helpup, 1889), Barbier, Herpin, Home (all three quoted by Sacher), Pera (1911), Lander and Edwards, etc.) has reported negative; another group (Orfila (quoted by Schlossberger), Werneck (1831), van Hasselt (quoted by Helpup), Michaelis, D'Amore and his associates, Hadlich (1908)), positive results following the taking of zinc oxide. Heller in 1847 reported that no symptoms followed the internal administration to man of zinc oxide in sugar solution in doses of 20, 60, and 120 grains a day—a result which led him to conclude that zinc oxide was physiologically inert and, therefore, useless as a medicine. Schlossberger wrote concerning "two people who, suffering from severe nervous troubles, gradually arrived in subsequent use of the metal (zinc oxide) at very strong doses, at which only with one of them sometimes slight vomiting appeared. . . . . The second patient was a student who on account of epileptic fits of many years' standing had taken zinc oxide many weeks."

In a discussion of the medicinal use of zinc oxide, Sacher referred to cases described by Barbier, by Herpin, and by Home in which doses of zinc oxide as large as 1.2 grams, 2.0 grams and 2.5 grams were taken in the course of a day without causing vomiting.

Wibmer (quoted by Helpup) gave a young dog from the middle of September to the middle of October, daily, 10 grains of zinc oxide. Appetite

and stools remained normal. The animal then tolerated to the end of October, 15 grains daily, consuming in six weeks an ounce of the oxide. Autopsy showed everything normal. Lander and Edwards in 1912 gave a calf and a full grown sheep a total of 2220 grains (148 grams) each, in the course of twenty-six days. For fifteen days each animal received 4 grams per day, and then for eleven days, 8 grams per day. No loss of appetite or condition—indeed no symptoms of any sort—were observed.

Pera, arguing in favor of zinc white as a substitute for lead white, cited the absence of toxicity as one of its advantages. He said that zinc oxide has been used as a teniafuge in a dose as large as 30 grams of zinc oxide powder in 60 grams of syrup for the first day and 15 grams the following day—and this without causing the least accident.

In contrast to these negative findings, we have in the literature accounts by a second group of investigators of active symptoms ensuing from the administration of zinc oxide. This group observed in man, burning in the stomach, eructations, griping, increased thirst, loss of appetite, vertigo, hot flashes, and general exhaustion following single doses of 6 to 8 grains of zinc oxide. With 10 grains, Werneck reported, in addition to these symptoms, sharp pains in the diaphragm and between the shoulders, later vomiting and the passage of a thin stool, with exhaustion lasting twenty-four hours.

The following protocol, taken from Michaelis, illustrates the type of result reported by the positive group of experimenters upon the administration of zinc oxide to animals.

A growing cat received in milk with white bread 10 grains of zinc oxide *via humida parata*. Restlessness, vomiting and diarrhea followed. There was much bile in the fecal masses. For three days the animal received on each day half the former dose. On the fourth day it received 6 grains, upon which the number of the stools increased. On the following day, 8 grains. The animal became restless, and devoured greedily what was thrown to it. In the afternoon there was retching and vomiting five times; at the end, bile-stained mucus. In the beginning, the stools were normal, later thin and fluid. On the following day, the animal was dull, apathetic, and ate first in the afternoon some food which had been mixed with 4 grains of zinc oxide. After a half hour the same disturbances as the day before appeared. The cat was very weak and could scarcely stand erect. Dose omitted on the seventh day. On the eighth day, 6 grains, loss of appetite and diarrhea. On the ninth day, perhaps 2 grains, which was soon got rid of; now there appeared a peculiar twitching of the limbs of the animal. It curved itself and stretched continually. Set the cat free and stretching continued. No desire to run away. This symptom developed on the next day into true cramps which attacked especially the hind extremities. The animal then fell on its side and went into violent twitchings, cried out and had tears in its eyes. After two minutes the attack ended and the animal was then free of the twitchings. The two following days, the same dose; no cramps. However, if a mouse was held before this animal, it at once developed vigorous cramps. Fourteen more days the animal received zinc; cramps were observed only once. Then it was killed after it had taken 92 grains (6+ gm.) of zinc oxide.

Autopsy finding: On the cardia two small erosions,—one on the greater curvature, everywhere else the stomach was normal. In the intestine there was much mucus and some reddening, beginning catarrh. Everything else was normal. In the liver 0.024 gm. of zinc oxide was found. Relatively much in the bile. Brain undetermined. Spinal cord showed nothing abnormal. In the urine during the entire time zinc was demonstrable.

The probability that the oxide used by Werneck, Michaelis, D'Amore, etc., contained some contaminating toxic substance is suggested by the conflicting evidence presented by other observers. This probability is converted, in our opinion, into a practical certainty when our own experience is brought into evidence.

EXPERIMENTAL METHODS. *Diet.* Besides the control animals, ten cats and three dogs were used in these investigations. The cats were kept in individual wire cages and were fed once a day canned salmon and milk. We have in the past in this laboratory kept cats fat and healthy under a similar régime for as long as two and a half to three years. The dogs were kept in individual cages at night, and in the daytime, except in very bad weather and on Sundays, were out-of-doors in an enclosed yard. The dogs were fed, daily, milk and a dog mash containing bread crumbs and ground-up meat. During a large part of the experimental period, they also received twice a week Hamburger steak and bones. We would like to emphasize here the importance of bones in the diet of a dog in captivity. During a part of the experimental period when the bones were omitted, the youngest of our dogs lost weight, although he was very lively and apparently perfectly healthy. As soon as bones were restored to the diet, his gain in weight was rapid.

Dosage. Zinc oxide was administered to these animals daily in the form of a dry powder mixed with the food. At the beginning of the experiments the cats were given, in milk, zinc oxide suspended in gum acacia, but this proved a less satisfactory method of dosage than to mix the dry powder with salmon.

Chemical analysis of the zinc oxide used gave the following results—figures which show it to be an extremely pure product:

	per cent
Insoluble in HCl	0.007
CO_2	0.025
Total S as SO_3	0.001
Cl	0.004
CdO	0.004
PbO	0.023
Bi_2O_3	under 0.001
CuO	under 0.001
Fe_2O_3	under 0.001
MnO	under 0.001
As_2O_3	under 0.001

Sb_2O_3	under 0.001
ZnO (diff.)	99.799
Red. Powder as SO_2	0.010
Water soluble salts dried at 110°C.	0.098

The amounts of zinc oxide administered daily to the cats ranged from 175 mgm. to 1 gram. The only unfavorable symptom which appeared in our animals was a lack of appetite in cats on large doses (above 600 mgm.), a symptom which we attribute to the disagreeably sweetish taste of the oxide-containing food, and not to nausea. We have had no difficulty at all with daily doses of 600 mgm. or less. Cats taking 200 to 600 mgm. ate heartily and regularly.

The dogs received 500 mgm. to 1 gram of zinc oxide daily mixed with food, and as a rule took it readily. The daily doses administered to the individual animals, in terms both of milligrams of zinc oxide and in milligrams of zinc per kilogram of animal, may be seen in table 1.

Methods of observation and collection of specimens. The animals were weighed weekly throughout the experimental period and were observed daily for signs of vomiting, diarrhea or other indication of illness.

Urine specimens were collected from the cats as follows: The animal was fed in the morning of the day preceding the collection of a 24-hour specimen. At 9 o'clock on the following morning, he was placed in a rectangular, oblong, copper-netting cage suspended over a large enamel pan, and left there with distilled water in a copper container to drink but no food. Under circumstances such as these, cats frequently neither void nor defecate for twenty-four hours. When such was the case, at the end of the twenty-four hours the animal was placed upon a cat board. Often the excitement incident on this caused him to void at once into a glass beaker held to the urethral opening. If not, we found it relatively easy to empty the bladder completely by grasping it through the abdominal wall and squeezing it gently and firmly. The maneuver resembles closely that applied to the uterus in expelling a retained placenta. An experienced person can readily tell by its small size when the bladder is completely empty. We could depend upon it that urine specimens obtained in this way were uncontaminated with particles of zinc oxide which might be upon the mouth or fur of the animals. Occasionally the cats urinated into the pan, but all pan urine specimens were discarded for fear of contamination. On a few occasions 24-hour fecal specimens were collected and analysed for zinc.

Urine specimens were collected from the dogs as follows: For the 24-hour period of the collection the dog was kept in a freshly washed cage. If at the end of the period he had neither urinated not defecated—and this was almost always the case—the dog was taken out-of-doors on a leash, and the urine, which the animal usually voided very promptly, collected

in an aluminum pan. *Feces* were collected similarly. At the beginning of the experiment the dogs were taught not to soil their cages, just as a pet dog is house broken, and they soon became accustomed not only to retaining urine and feces for a twenty-four hour period, but also to voiding and defecating at the end of this period into pans held in the proper position. The reasonable uniformity of volume of the specimens collected and of their zinc content have convinced us that our collections were complete and representative ones.

Urine specimens were always examined for albumin by the sulphosalicylic acid test; for sugar, with Benedict's reagent; and the sediment, for casts, pus cells, blood cells, etc.

Blood studies. Hemoglobin determinations and blood cell counts were made on each animal (except on three cats killed early in the series) just before it was killed for autopsy. Blood counts were also made on a few animals in the early part of the experimental period. More frequent blood studies were considered unnecessary because of the continuous good health of the animals.

Zinc dosage was discontinued in the case of four cats two weeks previous to autopsying them. Each one of these cats had an experimental mate which had been on the same zinc dose for the same period of time. Each of these latter animals was killed upon the same day that zinc dosing of its mate was discontinued. The object of this procedure was to determine how rapidly zinc which might be stored in the body would be eliminated.

Autopsies. The animals were killed at the end of the experiment by being bled to death under ether anesthesia, all the blood being saved for zinc analysis. Each animal was then skinned and the hide saved for analysis. Hides of zinced animals were washed with distilled water before ashing. Next, an autopsy was done, each organ being weighed, a section taken for histological study, and the rest saved for zinc determination. Large samples of muscle from the hind legs and of fat were taken for determination of zinc concentration in these tissues. In all cases, the gastro-intestinal tract from the pharynx down was slit open after removal from the animal and thoroughly washed with running water before being ashed. In the case of zinced animals, the mouth and pharynx were also thoroughly washed with distilled water as soon as the carcass was dismembered. In order to separate the bones from muscle, the carcass of each animal was cut up and boiled in distilled water for approximately six hours. The bones were then carefully picked out, ashed and analysed separately from the main body of muscle and connective tissue. This latter material was evaporated to dryness, ashed, and analysed. The zinc figures for muscle obtained in this way were checked by and averaged with analyses upon smaller samples of muscle cut from the thighs.

Chemical analyses. All tissues were dried, charred, and then ashed in an

electric muffle furnace at 450°C. The residues were extracted with 1:1 distilled hydrochloric acid and hot water, filtered, and the filter paper and contents re-ashed and extracted: this was repeated as often as was necessary for the complete destruction of all the organic matter.

Tissues were ashed and analysed in their entirety, except in the case of bones, carcasses, and dog hides, where fractions of total ash solution were taken for analysis, as follows:

	BONE		CARCASS		HIDE	
	Total volume	Volume analysed	Total volume	Volume analysed	Total volume	Volume analysed
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Cats	500	50	500	100		
Dogs	2000	100	1000	100	500	500

Iron was removed from the acid ash solutions with cupferron. The zinc and 2 to 5 mgm. of added copper were then precipitated together as sulphides in neutral or alkaline solution in the presence of a buffer salt; the precipitate was well washed with water and with hot alcohol; it was then dissolved in nitric acid, the latter fumed off, and the copper removed as sulphide in hydrochloric acid solution, leaving the zinc in pure form in the filtrate. The filtrate was next evaporated to dryness and made up to a known volume. For bones, it was necessary to use large volumes (600 to 800 cc.) when precipitating the zinc and copper together, and to repeat the precipitation, in order to eliminate calcium phosphate.

When the amount of zinc involved was in the range of 1 to 2 mgm. or less (e.g., urines and small organs), the final solutions were read by the ferrocyanide turbidimetric method. When the amount was over 1 to 2 mgm. (e.g., livers, bones, carcasses), the zinc was determined by the ferrocyanide titration method. The foregoing procedure is outlined in detail by Fairhall (1926a) in a paper published from this laboratory.

RESULTS OF CLINICAL AND LABORATORY OBSERVATIONS AND OF AUTOPSY FINDINGS. The clinical and laboratory observations and autopsy findings on the individual animals in this series of experiments are summarized in table 1. This table includes also the average daily dose of each of the animals and the duration of zinc administration.

General health. In the main, the health of all the animals was excellent throughout the experimental period. Several of the cats developed acute respiratory infections of varying degrees of severity—cat "snuffles"—during the course of the winter but all recovered satisfactorily. Spots of mange in a few of the animals, appearing early in the work, yielded readily to treatment. Dog 5, an animal in poor condition when we obtained her, very thin and with a limping gait, but chosen because of her relatively

TABLE 1

Summary of clinical and laboratory observations and of autopsy

ANIMAL NUMBER AND SEX	AVERAGE DAILY DOSE		DURATION OF ZINC ADMINISTRATION	WEIGHT		BLOOD FINDINGS				URINE FINDINGS*			
	Zinc oxide	Calculated as ingm. zinc per kgm. of animal		At beginning of zinc administration	At end of zinc administration	Week of observation	Hb	R.B.C.	W.B.C.	Number of specimens examined	Albumin	Sugar	Sediment
	mg.		*weeks*	*kgm.*	*kgm.*		*per cent*						
Cat 9♂ (castrated)	250	33.8	10 on zinc 2 off zinc	4.8	6.4 At end 2 weeks off zinc, 6.5	12th	80	11,089,000	25,000	7	Positive† (trace) twice in normal period. Negative thereafter	Negative throughout	Nothing significant
Cat 7♂	250	41.1	10	4.3	4.8	10th	100	12,096,000	32,900	8	Positive (trace) throughout both normal and zinc periods	Negative throughout	Nothing significant
Cat 3A♀	200	44.0	31	3.8	4.1	5th 31st	— 85	12,237,000 9,978,000	17,400 15,200	5	Positive (trace) throughout	Negative throughout	Nothing significant
Cat 2A♀	200	52.7	31 on zinc 2 off zinc	2.8	3.6 At end 2 weeks off zinc, 3.3	5th 33rd	— 95	9,121,000 10,122,000	20,500 30,100	8	Positive (trace) throughout	Negative throughout	Nothing significant
Cat 8♀	300	64.4	11	3.4	3.7	11th	75	9,264,000	18,800	6	Positive (trace) twice in normal period. Negative thereafter	Negative throughout	Nothing significant
Cat 10♂	300	66.2	11 on zinc 2 off zinc	3.0	4.1 At end 2 weeks off zinc, 4.1	13th	95	11,508,000	35,600	11	Positive (trace) throughout both normal and zinc periods	Negative throughout	Nothing significant

*Cats 7, 8, 9, and 10 and dogs 2, 4, and 5 each had three normal urine collections before being put on zinc.

†A trace

TABLE 1

Summary of autopsy findings upon experimentally zinced animals

	Sugar	Sediment	GENERAL HEALTH DURING EXPERIMENT	AUTOPSY FINDINGS
		Nothing significant	Health excellent throughout. Appetite good. No vomiting, diarrhea, muscular weakness, tremors, nor paralyses. Fur exceptionally thick and lustrous. See figure 1	All organs examined grossly and histologically and found to be normal
		Nothing significant	Same as above	Same as above
		Nothing significant	Same as above except for period of moderately severe respiratory infection, involving no weight loss, from which cat, to all outward appearances satisfactorily recovered	Only gross pathological finding was in posterior lobe of right lung where there was a small area containing a calcareous centre surrounded by a small amount of pus. All organs examined histologically and found to be normal except posterior lobe of right lung, which showed a patch of bronchopneumonia. Lung findings evidently incident to the respiratory infection, and not experimentally significant
		Nothing significant	Same as cat 3A. See figure 2	No gross pathological findings. All organs examined histologically and found to be normal except lungs which showed rather extensive peribronchial infiltration with lymphocytes, and a general increase in lymphatic tissue, finding not significant experimentally
		Nothing signficant	Four weeks after being put on zinc, 4 kittens were born to this cat. During week previous to their birth, she became severely excoriated around anus, a local irritation, unaccompanied by diarrhea or obstruction, which improved on treatment. A few days later she developed a very severe respiratory infection. After the kittens were born, the anal excoriation disappeared rapidly and there was a gradual recovery from the respiratory disease. Even though laboring under the double strain of nursing kittens and of the respiratory infection, this cat recovered remarkably well, and otherwise was in excellent health throughout the experiment	No gross pathological findings except a few small dark brown areas on outer surface of kidney. Examined histologically these proved to be very small highly cellular tumors, made up of large oval cells. Otherwise the kidney and all other organs were normal
		Nothing significant	Excellent throughout except for a mild respiratory infection during the 4th week of zincing	All organs examined grossly and histologically and found to be normal

† A trace of albumin in the urine of normal cats or dogs is a common finding.

ANIMAL NUMBER AND SEX	AVERAGE DAILY DOSE		DURATION OF ZINC ADMINISTRATION	WEIGHT		BLOOD FINDINGS				URINE FINDINGS*			
	Zinc oxide	Calculated as mgm. zinc per kgm. of animal		At beginning of zinc administration	At end of zinc administration	Week of observation	Hb	R.B.C.	W.B.C.	Number of specimens examined	Albumin	Sugar	Sediment
	mg.		*weeks*	*kgm.*	*kgm.*		*per cent*						
Cat 1 ♀	175 354	38.2 83.2	8 45 } 53	3.1	3.5	27th 53rd	— 85	10,152,000 11,711,000	32,300 16,700	7	A trace in three specimens	Negative throughout	Nothing significant
Cat 3 ♀	316 681	76.9 188.5	8 8 } 16	2.9	2.5					1	Positive (trace)	Negative	Nothing significant
Cat 4 ♂	614 1000	117.4 223.0	8 13 } 21 on zinc 2 off zinc	3.8	3.1					1	Positive (trace)	Negative	
Cat 5 ♂	627 1000	152.6 267.6	8 13 } 21	2.9	2.6					1	Positive	Negative	
Dog 4 ♂	500	36.1	19	9.9	11.9	3rd 19th	90 100	8,432,000 8,649,000	24,200 17,100	15‡	Positive (trace) in all but five, four of which were in zinc period	Negative except for one specimen in middle of zinc period	Always small bile-stained granular casts resent but not definitely fiable and casts
Dog 5 ♀	500	59.9	3	6.5	6.6	3rd	70	4,564,000	18,300	5	Four in normal period negative. The one specimen in zinc period +++ (one week before death)	Negative	Abundant pus cells 5th urine
Dog 2 ♀	1000	76.5	15	9.9	10.8	3rd 15th	90 100	9,176,000 8,835,000	20,900 19,100	14	Negative throughout both normal and zinc periods	Positive once in normal period and twice about middle of zinc period	Nothing significant

* Cats 7, 8, 9, and 10 and dogs 2, 4, and 5 each had three normal urine collections before being put on zinc.

‡ Thi

Continued

Sediment	GENERAL HEALTH DURING EXPERIMENT	AUTOPSY FINDINGS
Nothing significant	Health excellent throughout. There was occasional vomiting, but no more frequently than often occurs in a healthy pet cat during the course of a year. A small spot of mange, appearing over one eye, in the 11th month of the experiment yielded readily to treatment. See figure 3	All organs examined grossly and histologically and found to be normal
Nothing significant	Health excellent throughout. Occasional but not significant vomiting. This cat gained weight on the smaller dose but lost it on the larger one, apparently because the large dose of sweetish oxide rendered the food somewhat unpalatable and the cat, consequently, ate less than usual	Only gross pathological finding was the pancreas, which was markedly enlarged, hard in texture, and covered with nodules. All organs were examined histologically and found to be normal except the pancreas, which showed a generalized fibrous overgrowth affecting the entire organ. No evidence of any acute process
	Same as cat 3. An area of mange appeared on one leg but this responded readily to treatment	Same as for cat 3, except that grossly, the pancreas seemed neither so large nor so hard
	Same as cat 3. Severe mange appeared on one leg but disappeared readily under treatment	Grossly pancreas was enlarged, hard, and covered with nodules. Unfortunately no histological sections were made of the organs of this cat
Always showed bile-stained granular masses resembling but not definitely identifiable as cells and casts	Health excellent throughout. Appetite good. No intestinal nor nervous disturbances of any kind. He was sturdy and vigorous, with sleek and glossy coat. For a period during zinc administration, when bones were omitted from the diet, he lost weight, but when bones were included again, the loss in weight was fully and rapidly regained. See figure 4	All organs examined grossly and histologically and found to be normal
Abundance of pus cells in the 5th urine	This dog was in poor condition when we first obtained her. During the three weeks normal period preliminary to zincing, she improved markedly, gained nearly a kilo in weight and seemed quite lively and healthy, though when taken out on a leash she sometimes exhibited a peculiar sidling gait and was reluctant to run along with the other dogs. About ten days after zinc was begun she suddenly became utterly apathetic. This was her one outstanding clinical symptom. (Urine showed pus and albumin.) She practically refused to walk when taken out, although able to do so when compelled. There were no signs of intestinal disturbance, no discharges, no tremors nor paralyses. This lasted two days. In another couple of days she was apparently well. No relapses occurred. About a week later she was found dead (for some hours) in the yard without gross evidence as to the cause of death	Only gross pathological finding was slight infection of the meninges. All organs were examined histologically. The kidney showed swelling and overfilling of kidney vessels. The lung showed an exudate: much fibrin, many leucocytes: but no definite solidification. Other organs were normal. Diagnosis: *Distemper pneumonia*
Nothing significant	Same as dog 4, except that there was no weight loss when bones were omitted from the diet, a difference in reaction probably due to the fact that dog 4 was a young growing dog whereas dog 2 was adult	All organs were examined grossly and histologically and found to be normal. Five very young embryos were found

‡ This dog's urine always contained readily detectable bile.

small size, died of distemper three weeks after the beginning of zincing. Lungs, kidneys and meninges were involved in this infection, the dog finally succumbing to distemper pneumonia.

With these exceptions, our animals were an unusually healthy group. There was no significant vomiting nor diarrhea, no muscular weakness,

Fig. 1. Cat 9. Dose, 250 mgm. zinc oxide daily for ten weeks. Photograph taken at end of tenth week of zincing.

Fig. 2. Cat 2A. Dose, 200 mgm. zinc oxide daily for thirty-one weeks. Photograph taken at end of thirty-first week of zincing.

Fig. 3. Cat 1. Dose, 175 mgm. zinc oxide daily for eight weeks and then 354 mgm. daily for forty-five weeks. Photograph taken at end of forty-fifth week of zincing.

Fig. 4. Dog 4. Dose, 500 mgm. zinc oxide daily for nineteen weeks. Photograph taken at end of ninteenth week of zincing.

tremors, nor paralyses. The fur of the cats was exceptionally sleek, thick and lustrous; indeed, these animals were frequently shown to visitors, not as examples of the non-toxicity of zinc oxide, but as proof of the excellent health and condition in which it is possible to maintain cats in captivity over long periods of time when proper diet and housing conditions are provided.

Figures 1, 2, 3 and 4 are photographs illustrating the general healthy appearance which all of our experimental animals presented.

Weight. With the exception of three cats (nos. 3, 4 and 5) on a very heavy daily dose of zinc oxide—approximately 600 to 1000 mgm.—an amount of sweetish powder so large as to make the salmon unpalatable, all of the animals gained weight, some of them markedly, during the weeks of zincing. The three cats in question lost during sixteen, twenty-one, and twenty-one weeks of zinc, respectively, 0.4, 0.7 and 0.3 kgm.—losses not large enough to be significant. These highly zinced cats exhibited occasional vomiting, but this was neither striking nor consequential, being no more frequent than often occurs in a normal pet cat during a period of months. There was no diarrhea nor other significant evidence of digestive disturbance. Lack of appetite was the only noticeable symptom, and this we attributed to the unpalatability of the food. When one considers that these three cats were receiving during the period of lack of appetite, a daily dose of zinc oxide equivalent, in terms of a man of 70 kgm., to amounts ranging approximately between 14 and 23 grams per day, one is only surprised that they ate at all.

This excessive dose was given to a few animals, not because it was considered especially useful to determine the physiological effects of huge amounts of zinc, but rather to determine whether or not the normal zinc content of the body could be significantly increased by zinc administration, and, if so, whether or not abnormal deposits of zinc would be retained when dosing was discontinued. The results, from this point of view, will be discussed under the question of zinc storage.

Blood findings. With the exception of three cats (nos. 3, 4 and 5) which were killed first in the series and before this procedure was decided upon, blood counts and hemoglobin determinations were made upon all of the animals on the last day of the experiment. In a few instances counts were also made after a few weeks of zincing. The individual findings are given in table 1. The red cell counts (ten observations) in the cats averaged 10,728,000; the white counts, 24,500; and the hemoglobin, 88 per cent. In the dogs, the averages were as follows: red cells, 7,931,000; white cells, 19,900; hemoglobin, 90 per cent. With the exception of one red count on the dog that died of distemper (no. 5), the red cell counts on both cats and dogs were in the high range or somewhat above the high normal levels reported by other observers. A comparsion of the extreme red cell counts

reported by other observers upon healthy cats and dogs with the extremes obtained by us upon zinced cats and dogs is as follows:

Cats

	R. B. C.
Goodall (1909)	7,280,000 to 8,690,000
Wells and Sutton (1915)	9,628,000 to 9,664,000
Zinced cats	9,121,000 to 12,237,000

Dogs (non-pregnant)

	R. B. C.
Dawson (1930)	6,250,000 to 8,500,000
Goodall	4,750,000 to 5,500,000
Musser and Krumbhaar (1914)	4,630,000 to 7,760,000
Wells and Sutton	6,593,690 to 7,690,000
Zinced dogs	4,564,000 to 8,835,000

These figures indicate clearly that the daily ingestion of considerable quantities of zinc by cats and dogs over long periods of time does not cause anemia in these animals.

Our white counts, though apparently high on first inspection, correspond closely to the normal figures reported by various observers. The same is true of the hemoglobin determinations. High and low normal white counts and hemoglobin determinations taken from the literature as compared with our figures are as follows:

CATS	W.B.C.	PER CENT Hb
Wells and Sutton	9,000 to 20,600	
Goodall	15,800 to 38,800	65 to 80
Zinced cats	15,200 to 35,700	75 to 100
DOGS	**W.B.C.**	**PER CENT Hb**
Dawson	11,000 to 28,000	59 to 98
Musser and Krumbhaar	8,800 to 33,050	81 to 110
Goodall	11,200 to 31,200	75 to 110
Zinced dogs	17,100 to 24,200	70 to 100

Clearly, the zinc has had no deleterious effect on the blood picture of our animals.

Urine findings. From dog 2, an animal receiving a daily dose of 1 gram of zinc oxide, fourteen specimens were collected (four controls, ten specimens during the zincing period) and all were negative for albumin with the sulphosalicylic acid test. This was the only animal, the urine of which was uniformly negative for albumin throughout both normal and zinc periods. Cats 8 and 9 showed traces of albumin in the normal period but none in the zinc period. Cats 7 and 10 showed a trace of albumin through-

out both normal and zinc periods. Control specimens of urine were, unfortunately, not obtained from cats 1, 3, 4, 5, 2A and 3A, and, with the exception of cat 1, in which a trace of albumin was found in three out of seven specimens, all of these animals showed positive sulphosalicylic acid albumin tests in all specimens collected during the zincing period. The complete absence, however, of significant findings, such as casts, white or red cells, in the sediment of the cat urines; the minute amounts of albumin detectable by the sulphosalicylic acid test; the common occurrence of small amounts of albumin in the urine of normal cats and dogs, as indicated both in the control specimens from our zinced animals and in the eight specimens collected from control cat 6, all of which were positive; and, finally, the normal kidney findings in all of the animals (except in dog 5)—these facts all indicate that the traces of albumin found in the urines of our zinced cats have no special pathological significance.

Of fifteen specimens from dog 4, four out of five were positive for albumin in the normal period, and six out of ten in the zinc period. Curiously enough, the urine of this animal in both control and zinc periods always contained bile and the sediment showed bile-stained granular masses resembling, but not definitely identifiable as cells and casts. As is so common in male dogs, a drop of pus was always evident on the tip of this dog's penis. The possibility that some sort of chronic genito-urinary infection was responsible for some of the urine findings is worthy of consideration. In any event, histological examination after death, of the liver and kidneys of this animal showed these organs to be entirely normal.

Four control urines from dog 5 were negative; one specimen collected during the zincing period, one week before her death from distemper, contained marked albumin and an abundance of pus cells—a finding which we attribute to an involvement of her kidneys in the acute intercurrent infection of which she died.

All urines of all cats were negative throughout for sugar (Benedict test). Dog 2 gave a positive Benedict once in the control period and twice about the middle of the zinc period, and dog 4 once in the middle of the zinc period. Histological examination showed normal kidneys and normal pancreases in both of these animals.

Autopsy findings. The details of the gross autopsy and of the histological findings upon all of the animals are given in detail in table 1. Two of the cats showed small pathological areas in the lungs resulting from intercurrent respiratory infection not completely resolved. The kidney of one cat showed, grossly, a few dark brown areas on the outer surface, which proved on histological examination to be small highly cellular tumors. The kidneys of dog 5 who died of distemper pneumonia showed swelling and overfilling of the vessels; the lungs of this animal showed an exudate, much fibrin, and many leucocytes, but no definite solidification. The other organs were normal.

With the exception of these findings, which were incidental and without experimental significance, and of fibrotic changes in the pancreases of three cats—an observation discussed in the next paragraph—all of the organs (stomach, duodenum, liver, spleen, lungs, kidneys, adrenals, reproductive glands, submaxillary gland, thyroid) were examined grossly and histologically and were found to be normal. Three cats (nos. 3, 4 and 5), on extremely large daily doses of zinc oxide (maximum, 681 to 1000 mgm.), showed at autopsy pancreases which grossly appeared harder than normal and were covered with firm nodules. Through a misunderstanding, the organs of cat 5, the first animal in the series killed, were taken in their entirety for chemical examination and no portions were saved for histological study. Presumably, however, the microscopic findings in the pancreas would have been similar to those in cats 3 and 4, since the gross appearance of each of the three organs was similar. The weight of cat 5's pancreas was 3 grams—a rather low figure. The histological reports on cats 3 and 4 are as follows:

Cat 3: Pancreas: Weight, 10 grams. Perfect acini are not common. The tissue is heavily and diffusely overgrown with fibrous elements. Fibrous trabeculae are wide and heavy and extend diffusely into the groups of acini, sometimes apparently abolishing them. No definite islets are made out. There is no distension of ducts and no evidence of any acute process—simply a generalized fibrous overgrowth affecting the entire organ.

Other organs normal.

Cat 4: Killed two weeks after discontinuance of zinc administration. *Pancreas:* Weight 3.8 grams. The gland is very firm but not so hard nor so large as the pancreas of cat 3, an animal killed during zinc administration. Histological examination shows many normal acini which are, however, sewn through with large amounts of fibrous tissue. Here and there, one can make out groups of cells constituting islands of Langerhans, but these are not very well defined. There is no hemorrhage, merely a considerable proliferation of fibrous tissue all through the stroma of the gland.

Other organs normal.

Except in the pancreases from cats 3, 4 and 5—animals receiving doses of zinc oxide far beyond any conceivable human exposure—we have never found histological evidence of pancreatic damage in zinced animals, though the number of zinced animals which we have studied in this connection includes forty individuals (rats, kittens, cats and dogs). Further discussion of the pancreatic findings in cats 3, 4 and 5 occurs in the section on zinc excretion.

Summarizing our clinical and laboratory observations and our autopsy findings upon zinced animals, we may say that we have never observed any significant clinical symptom nor obtained any significant laboratory evidence of damage resulting from the daily ingestion of zinc oxide over long periods of time. With the exception of fibrous change in the pancreases of three excessively zinced cats, histological examination following autopsy

has shown in the organs of zinced cats and dogs no damage of any sort attributable to zinc.

It is worth noting again at this point that sugar was uniformly absent from the urine of all our zinced cats, including specimens from the three

TABLE 2

Normal zinc content of urine and feces of cats and dogs

ANIMAL	DATE	URINE				FECES: ZINC CONTENT PER 24 HOURS
		24-HOUR VOLUME	Zinc content			
			Per 24 hours	Per cc. urine,	Per kgm. animal	
	1925	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Cat 6	December 7	27	0.28	0.010	0.06	4.4
Cat 7	November 5	81	0.43	0.005	0.09	
	November 11	64	0.21	0.003	0.05	
Cat 8	November 4	64	0.11	0.002	0.04	
	November 9	36	0.14	0.004	0.04	
	November 14	86	0.06	0.001	0.02	
Cat 10	October 31	63	0.11	0.002	0.04	
	November 13	72	0.07	0.001	0.02	
Average			0.18	0.004	0.045	
Dog 2	November 21	80	0.38	0.005	0.038	
	December 4	80	0.19	0.002	0.020	25.2
	December 9	89.5	0.41	0.005	0.042	5.2
	December 15	73	0.33	0.005	0.033	
Dog 3	December 1	110.5	0.25	0.002	0.022	
	December 5	121.5	0.40	0.003	0.034	
	December 9	104.5	0.39	0.004	0.033	
	December 15	80.5	0.13	0.002	0.011	
Dog 4	November 20	195	0.55	0.003	0.064	17.6
	December 2	60	0.28	0.005	0.031	22.0
	December 9	154.5	0.59	0.004	0.060	4.2
Dog 5	November 25	100.5	0.32	0.003	0.054	
	December 9	71.5	0.37	0.005	0.056	8.0
Average			0.35	0.004	0.041	

animals with the fibrosed pancreases. Two dogs, one on one occasion, the other twice, passed urine specimens during the zinc period which were positive for sugar. One control specimen from the second dog, however,

was also positive, and both of these dogs showed normal pancreases at autopsy. The production of glycosuria by the administration of zinc acetate and malate, particularly when given intravenously, was reported in 1918 by Salant and Wise. These authors attributed the glycosuria, not to the pancreas, but to an increased permeability of the kidneys to sugar and designated the condition as a renal diabetes. Nearly thirty years previously, D'Amore and his associates reported glycosuria in their zinc oxide-fed dogs, but the probability of lead or arsenic contamination of the oxide used by these investigators leads us to discard this observation along with their other findings. Certainly, further experimental evidence is required before an association may be made between zinc and diminished pancreatic function.

ZINC EXCRETION. *Zinc in normal cat and dog urine.* The normal amount of zinc in cat urine, as indicated by our series, ranges between 0.06 and 0.43 mgm. per twenty-four hours, and in dog urine between 0.13 and 0.59 mgm., the averages for these two species of animal being 0.18 and 0.35 mgm. of zinc per twenty-four hours, respectively. Table 2 gives a series of zinc analyses upon the urines of normal cats and dogs. This detail of figures is presented because there exist in the literature no standards of normal zinc excretion in these animals.

Zinc in normal cat and dog feces. One 24-hour specimen of normal cat feces showed 4.4 mgm. of zinc while six specimens of normal dog feces showed, respectively: 25.2, 5.2, 17.6, 22, 4.2 and 8 mgm. (table 2). These figures indicate a range in the fecal zinc content of dogs very similar to that found in men on an ordinary mixed diet—i.e., approximately 3 to 20 mgm. per twenty-four hours (Drinker, Fehnel and Marsh, 1927).

Zinc in urine of zinc-fed animals. Four cats, upon daily doses of zinc oxide which ranged between 200 and 300 mgm., were included in this group, and three dogs, one of which received 1 gram of zinc oxide daily, the other two, ½ gram. The average amount of zinc excreted in zinc feeding is contrasted in figure 5 with the average daily normal urinary zinc excretion, and, in the case of the cats, with the average amount of zinc in 24-hour specimens of urine obtained from the zinced animals during a period of two weeks immediately following the discontinuance of zinc dosing. In order to show sample series of figures from which these averages were developed, the complete data upon cat 10 and dog 4 are presented in table 3, and are charted also in figure 5.

These figures show that during periods of zinc oxide feeding the amounts of zinc in the urines of cats and dogs increase markedly, and that, when zinc dosage is discontinued, urinary zinc rapidly falls to the normal level. In view of the absence of abnormal clinical findings in the urine of our zinced animals during life, and of the normal histological findings in the kidneys of these animals following autopsy, this marked increase in urinary

zinc during zinc feeding is a point especially to be noted. Batchelor, Fehnel, Thomson, and Drinker (1926) observed the same thing in their studies upon zinc excretion among zinc workmen—i.e., abnormal amounts of zinc in the urine for periods of many years, but unassociated with evidence of kidney damage.

The rapid disappearance of abnormal amounts of zinc from the urine when zinc feeding is discontinued suggests that significant abnormal

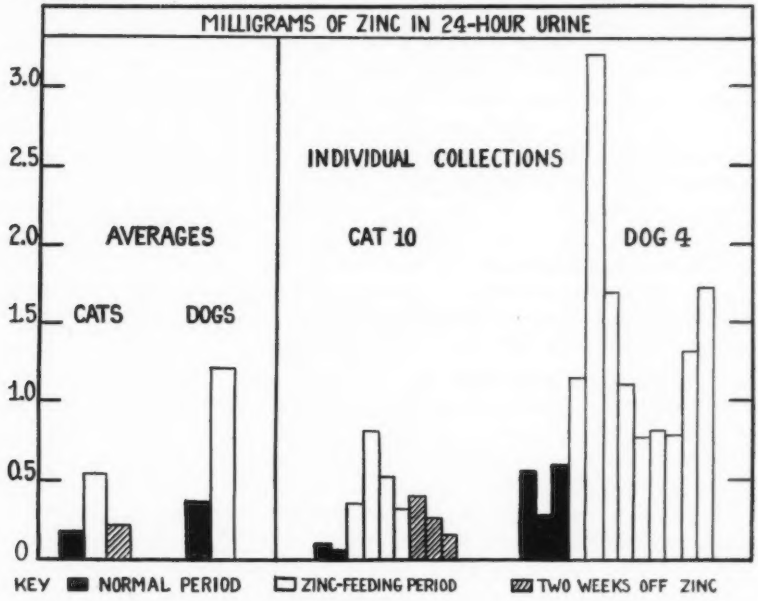

Fig. 5. Average 24-hour excretion of zinc in the urine of normal cats and dogs contrasted with the amounts of zinc excreted in the urine of the same animals during zinc feeding, and, in the cats during a 2-week period following the discontinuance of zinc feeding. Note the fall of urinary zinc to a normal level when zinc dosing is discontinued. Zinc in individual specimens from cat 10 and from dog 4 during control and experimental periods is also plotted to show 24-hourly variations in zinc excretion in spite of constant dose.

amounts of zinc are not stored in the body, but that zinc in excess of the normal body requirement is excreted approximately as rapidly as it is taken in. We have not been able to correlate the amounts of zinc in the urine with the size of the zinc dose. Dog 2, for example, on a daily dose of 1 gram of zinc oxide, excreted, as a rule, less zinc in the urine than dog 4 on a daily dose of ½ gram.

Zinc in feces of zinc-fed animals. Analyses were not made routinely

upon the feces of zinc-fed animals because, in our opinion, the results would be of little value since it would be impossible to determine how much of the zinc recovered represented zinc which had been absorbed and subsequently excreted, and how much of it represented zinc which had passed through the intestinal tract unabsorbed. A single analysis of fecal zinc

TABLE 3

Zinc in urines of cat 10 and dog 4 during control periods, during zinc feeding, and in cat 10 during two weeks following discontinuance of zinc feeding

ANIMAL	PERIOD	DATE	URINE		
			24-HOUR VOLUME	Zinc	
				In 24 hours	Per cc.
			cc.	*mgm.*	*mgm.*
Cat 10	Normal	October 31	63	0.11	0.002
		November 13	72	0.07	0.001
	Zinc feeding November 25, 1925 to February 5, 1926 300 mgm. ZnO daily	December 23	42	0.35	0.008
		January 7	52	0.80	0.015
		January 16	47	0.52	0.011
		January 27	40	0.32	0.008
	Off zinc	February 6	71	0.40	0.005
		February 12	100	0.26	0.003
		February 16	50	0.16	0.003
Dog 4	Normal	November 20	195	0.55	0.003
		December 2	60	0.28	0.005
		December 9	154.5	0.59	0.004
	Zinc feeding December 17, 1925 to April 30, 1926 0.5 gram ZnO daily	December 30	114	1.14	0.010
		January 15	122	3.20	0.026
		January 27	80	1.68	0.021
		February 6	135	1.10	0.008
		February 13	125	0.77	0.006
		March 3	95	0.81	0.009
		March 12	150	0.78	0.005
		March 30	116	1.32	0.011
		April 27	270	1.72	0.006

made on a 24-hour specimen from cat 9 (daily dose, 250 mgm. zinc oxide) gave 265.8 mgm. zinc, a figure which indicates, obviously, that ingested zinc leaves the body in major part in the feces. No special significance need be attached to the fact that in this particular instance, the zinc output for one day was greater than the daily dose. Our experience with

rats[2] indicates that a comparison of daily zinc intake with daily zinc output will reveal small lags and small spurts in zinc excretion, but that, when a comparison of the two processes over a long period of time is made, they will be found to be in an approximate equilibrium.

Pancreas as a possible factor in excretion of zinc. The fibrous changes observed in the pancreases of three excessively zinced cats (nos. 3, 4 and 5) have already been discussed. When analysed chemically, the three pancreases in question yielded amounts of zinc greatly in excess of the normal. The normal concentration of zinc in cat pancreas (see table 4) is 0.026 mgm. per gram of tissue. The concentrations in the pancreases of cats 3, 4 and 5 were, respectively, 0.410, 0.233, 0.260. Our less heavily zinced cats and dogs, when killed during the course of zinc administration, also showed a slight increase of zinc in the pancreas. At the end of two weeks following the discontinuance of zinc, the zinc content of the pancreas, in three cats subjected to this procedure, had, however, fallen practically to a normal level.

These observations, together with some unpublished ones, in which the zinc content of pancreases of animals subjected to the inhalation of zinc was found to double or even treble within three hours and to reach the high levels of cats 3, 4, and 5 within one to four days following exposure to zinc fume, have lead us to conceive of the possibility that the pancreas may serve normally as one of the agents by which zinc, or possibly other heavy metals, are excreted into the gastro-intestinal tract, and that the fibrosis in the cases under discussion may have resulted from an excessive demand upon the pancreas to exercise this function.

This suggestion is obviously a tentative one, and depends for its proof upon confirmation of Delezenne's finding of zinc in normal pancreatic juice, upon the establishment of normal standards of zinc content in this fluid, and upon the recovery of abnormal amounts of zinc in the pancreatic juice of zinced animals—an experiment which we plan to undertake at a future time. We are the more inclined to venture this hypothesis in regard to the pancreas because of the fact that, with the exception of the pancreas and the bone, the only organs which show any significant increase in zinc concentration following zinc feeding (see figs. 6 and 7), are organs taking part in the excretion of this metal,—viz., liver, gall bladder and bile (analysed together), gastro-intestinal tract, and kidney. The zinc concentration of bone falls to a normal level in cats on discontinuance of zinc feeding (see fig. 6), while that of the pancreas, like the liver, falls—but not within two weeks to the normal level—thus suggesting an excretory mechanism still at work.

In summary, we may say that our evidence in regard to the excretion of

[2] Unpublished data.

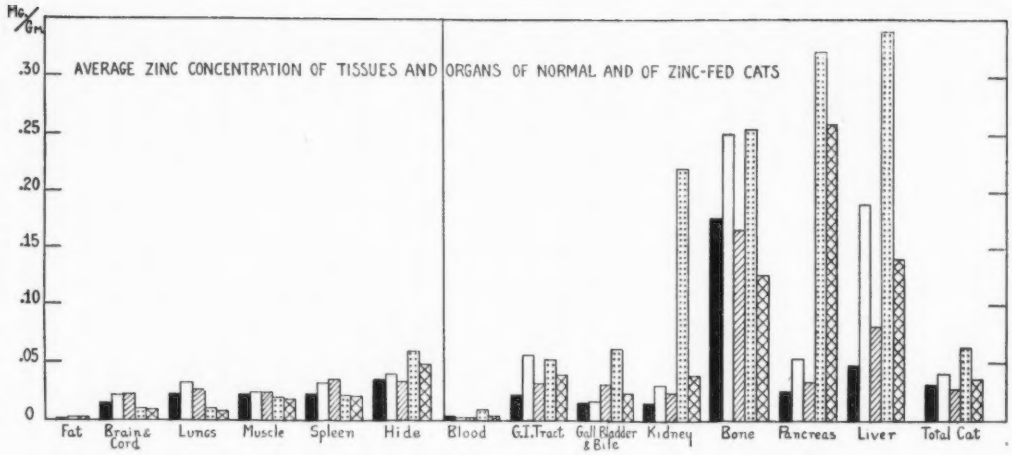

Fig. 6. Average zinc concentration of tissues and organs of normal cats (black columns); of cats receiving 200 to 354 mgm. zinc oxide daily and killed at height of zinc administration (white columns); of cats receiving similar doses for similar periods of time but not killed until two weeks after discontinuance of zinc dosing (single cross-hatched columns); of cats receiving excessively large doses of zinc oxide (681 to 1000 mgm.) and killed during zinc administration (dotted columns); and of one cat receiving a similar dose for a similar period of time but not killed until two weeks after discontinuance of zinc (double cross-hatched columns). See text for comment on noteworthy points.

zinc confirms that in the literature,—namely, that a small fraction of absorbed zinc leaves the body in the urine, but that the main bulk of it is excreted into the alimentary tract—some of it directly, some of it by the liver into the bile, and possibly some of it by the pancreas—and ultimately leaves the body in the feces.

ZINC STORAGE. *Zinc concentrations in tissues of normal cats and dogs.* Table 4 contains data upon the normal zinc concentration in the tissues and organs of cats and dogs. These figures include average normal concentrations of cat organs and tissues obtained by Lutz (1926) in this laboratory with his urobilin method (Lutz, 1925). Using the turbidimetric method of zinc analysis, we have, in a few instances (notably, gall bladder and bile, spleen, submaxillary gland, bone, brain and cord), obtained somewhat higher results than those reported by Lutz. In the main, however, our

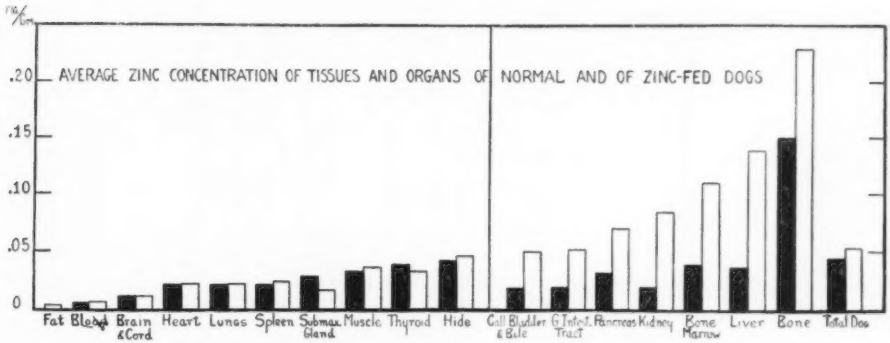

Fig. 7. Average zinc concentrations of tissues and organs of normal dogs (black columns) plotted against average zinc concentrations of tissues and organs of zinc-fed dogs (white columns). See text for comment on significant points.

figures and those of Lutz closely conform. In plotting normal zinc concentrations in cat tissue we have used an average normal obtained by averaging our normal figure with Lutz's average for the same tissue. The data upon normal dogs, however, are entirely new and are presented in detail (table 4) because of the absence in the literature of adequate normal standards of zinc concentration in dog tissues, and because of their usefulness in investigations not only upon the physiological action of abnormal amounts of zinc but upon the normal zinc metabolism as well. The percentage distribution of the total zinc content of a normal dog is plotted in figure 8. Of a total of 556.55 mgm. of zinc recovered from this animal, approximately 52 per cent was localized in muscle, 26 per cent in bone, and 13 per cent in hide.

Zinc concentrations in tissues of zinced animals. Ten cats and three dogs

TABLE 4

The normal concentration of zinc in the tissues of cats and dogs, expressed in milligrams of zinc per gram of tissue

ANIMAL	ADRENALS	BLOOD	BONE	BONE MARROW	BRAIN AND CORD	FAT	GALL BLADDER AND BILE	GASTRO-INTESTINAL TRACT	HEART	HIDE	KIDNEY	LIVER	LUNGS	MESENTERIC LYMPH GLANDS	MUSCLE	PANCREAS	REPRODUCTIVE TRACT		SPLEEN	SUBMAXILLARY GLANDS	THYROIDS	TOTAL ANIMAL
																	Ovaries	Testes				
Cats (♂):																						
Lutz' (1926) average.....	*	0.005	0.126		0.009		0.006	0.019		0.039	0.014	0.041	0.015		0.021	0.025		*	0.013	0.010	0.038	0.033
Our average..	*	0.002	0.227		0.020	0.001	0.024	0.025		0.030	0.013	0.055	0.028	0.016	0.022	0.027		*	0.033	0.038	0.052	0.031
Average.....	*	0.004	0.177		0.015	0.001	0.015	0.022		0.035	0.014	0.048	0.022	0.016	0.022	0.026		*	0.023	0.024	0.045	0 032
Dogs:																						
Dog 1♀.....	0.016		0.11		0.009		0.014	0.013	0.024		0.011	0.040			0.036	0.021	0.034		0.015			
Dog 3♀.....	0.046	0.003	0.19		0.012		0.009	0.024	0.017	0.043	0.034	0.033	0.026		0.030	0.034	0.120		0.016			0.045
Dog 6♂.....	0.071	0.006		0.038			0.030				0.012		0.015	0.036		0.041		0.023	0.032	0.028	0.039	
Average.....	*	0.005	0.15	0.038	0.011		0.018	0.019	0.021	0.043	0.019	0.037	0.021	0.036	0.033	0.032	*	*	0.021	0.028	0.039	0.045

* Average not significant because of wide range of concentrations in different individuals: e.g., cat adrenals, 0.033–0.194; cat testes, 0.008–0.046.

are included in this section, the daily doses of zinc oxide varying from 200 to 1000 mgm. and the duration of zinc administration from three to fifty-three weeks. For convenience in analysis, the cats have been divided into four groups—i.e., four cats (nos. 1, 8, 7 and 3A, see table 1) receiving large daily doses of zinc oxide (200 to 354 mgm.) and killed at the height of zinc administration; three mate cats (nos. 10, 9 and 2A) receiving similar doses for similar periods of time but not killed until two weeks after discontinuance of zinc administration; two cats (nos. 3 and 5) receiving excessively large daily doses of zinc oxide (681 to 1000 mgm.) and killed during zinc

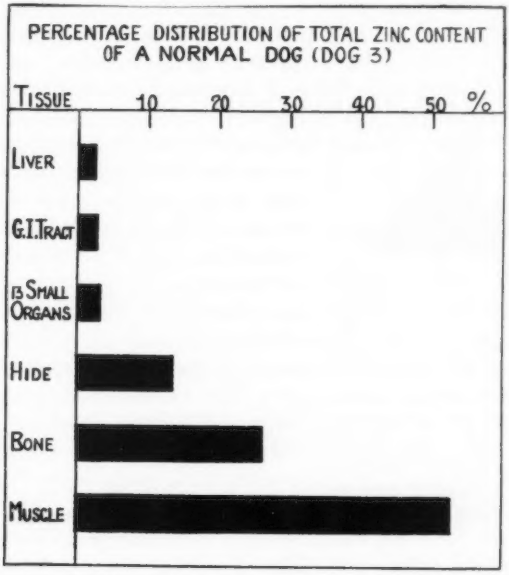

Fig. 8. Percentage distribution of the total zinc content (556.55 mgm.) of a normal dog (dog 3).

administration; one cat (no. 4), a mate to cat 5, receiving a similar dose for a similar period of time but not killed until two weeks after the discontinuance of zinc dosing. Details as to size and duration of dose for individual animals may be obtained from table 1.

The average zinc concentrations in the organs and tissues of the cats in each of these groups is plotted in figure 6 against the average normal zinc concentration of cat tissues and organs. Our studies upon zinc storage include, besides these cats, three dogs, two of which received $\frac{1}{2}$ gram of zinc oxide daily for three weeks (dog 5) and for nineteen weeks (dog 4), respectively, and one which received 1 gram daily for fifteen weeks (dog 2).

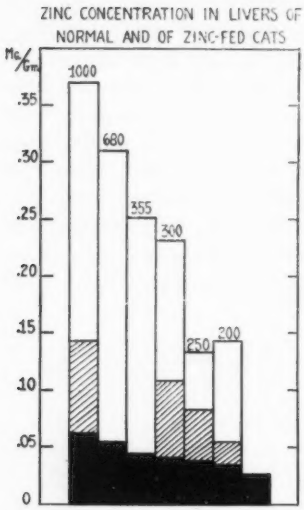

Fig. 9. Zinc concentrations of livers of six zinc-fed cats (200 to 1000 mgm. zinc oxide daily) killed at height of zinc administration (white columns), and of four cats on similar doses for similar periods of time but killed two weeks after discontinuance of zinc dosing (cross-hatched columns), plotted against the zinc concentration of the livers of seven normal cats (black columns). Note the proportion between size of dose and zinc concentration in the liver, and the rapid fall in liver zinc concentration when zinc dosing is discontinued. The numbers at the tops of the columns indicate daily zinc doses in milligrams of zinc oxide.

Dogs 2 and 4 experienced no ill health and were killed the day following the last dose of zinc. Dog 5 died of an intercurrent distemper pneumonia after only three weeks of zincing. The average concentrations of zinc in the organs of these three dogs are plotted in figure 7 against the average normal zinc concentration of dog tissues and organs.

The significant facts upon zinc storage brought out in figures 6 and 7 may be summed up as follows:

a. The ingestion of zinc oxide over long periods of time, even in very large amounts, does not increase the zinc concentration of fat, brain and cord, heart, hide, lungs, muscle,[3] spleen, submaxillary or thyroid glands. The slight apparent increase in cat hide concentrations shown in figure 6 in the heavily zinced cats is so probably due to slight contamination of the fur with zinc oxide of the dose that we attribute no significance to it.

b. Zinc concentration in the blood of cats and dogs is not significantly increased unless the amount of zinc oxide ingested be excessively large, and a blood increase depending on excessive ingestion rapidly falls to a normal level when zinc dosing is discontinued.

c. The zinc concentrations of organs involved in the excretion of zinc—i.e., liver, gall bladder with its content of bile, gastro-intestinal tract and kidney—are increased as a result of zinc oxide ingestion, the increase in general varying

[3] The figures for muscle used in the charts were derived by averaging the zinc figure obtained from analysis of an approximately 100-gram sample of pure muscle of a particular animal with the figure obtained from the analysis of the great mass of muscle, ligaments, and bits of fat left after complete removal of organs and bones (following prolonged boiling in water). The zinc concentrations of pure muscle and of this muscle mass were so nearly identical as to make this procedure a justifiable one.

directly with the size of the dose. The zinc concentrations in these organs, however, fall rapidly to or towards the normal level when zinc administration is discontinued. This fact is graphically shown in figure 9, in which are plotted the zinc concentrations of the livers of ten zinc-fed cats (four of them off zinc for two weeks) against normal zinc figures for cat liver (seven observations).

d. The zinc concentration of the pancreas is increased by zinc feeding, the increase being greatest in the animals receiving excessive doses, but falls off on discontinuance of zinc administration. A possible excretory function of the pancreas is suggested in this connection.

e. Bone marrow in dogs (not analysed in cats) and total bone in both cats and dogs show, as a result of zinc feeding, an increase in zinc concentration. This increase in bone returns to normal when zinc is discontinued.

f. The total zinc concentration of zinc-fed animals increases slightly with moderate zinc dosing and somewhat more markedly with excessive dosing, but falls to a normal level when zinc dosing is discontinued. This fact is a striking and significant one.

The zinc concentrations of the adrenals and of the sex glands in the various control and experimental animals are not charted because the normal figures for these organs, as well as the figures from the zinced animals, show such great variability as to suggest that the zinc content of these glands may be dependent upon functional activity,[4] varying with the state of the gland at the time it happens to be taken for analysis. The following figures illustrate, for example, the variable zinc concentrations found in the adrenals of different animals:

	Mgm. of zinc per gram of tissue
Normal control male, cat 6	0.194
Castrated male, zinced cat 9	0.020
Female, zinced cat 1	0.166
Male, zinced cat 10	0.268
3-weeks old kitten (nursing zinc-fed mother)	0.750
8-weeks old kitten	0.470

The following are a few figures upon the zinc content of ovaries of different zinced cats. Unfortunately we have no normal ovarian figures.

	Mgm. of zinc per gram of tissue
Cat 1	0.273
Cat 3A	0.077
Cat 2A	0.523*
Cat 8	0.370
Cat 3	0.036†

* Mate to 3A and off zinc for two weeks. Note, however, the much higher zinc figure.

† Excessively zinced cat.

[4] For discussion of zinc in its relation to functional activity, see Drinker and Collier (1926).

In a consideration of figure 7, plotted from analyses of the organs of zinced dogs 2, 4 and 5, it is worthy of comment that the zinc concentrations of the bone, bone marrow, combined gall bladder and bile, gastro-intestinal tract, hide, kidneys and liver in dog 5 were markedly higher than the zinc concentrations in the same organs of dogs 2 and 4, both of which had ingested zinc, and dog 2 in double the dose, for a much longer period of time. The contrasting figures in milligrams per gram of tissue are as follows (dog 5 versus the average of dogs 2 and 4): bone marrow, 0.26 *vs.* 0.035; bone, 0.28 *vs.* 0.21; gall bladder and bile, 0.076 *vs.* 0.050; gastro-intestinal tract, 0.075 *vs.* 0.040; hide, 0.063 *vs.* 0.039; kidney, 0.183 *vs.* 0.035; liver, 0.224 *vs.* 0.097; total dog, 0.068 *vs.* 0.046.

Three possible explanations of these higher zinc concentrations in dog 5 present themselves: the possible existence of exceptional animals with a greater tendency than the average to store zinc; the possibility that the normal tendency to excrete zinc is interrupted or impeded by intercurrent disease or that a leucocytic infiltration of the affected parts increases their zinc content (cf. Delezenne's finding of high zinc content in leucocytes); or, to our minds, the much more probable explanation that, when an animal's zinc intake is markedly increased, a definite interval of time is required for an approximate equilibrium between zinc intake and zinc excretion to be established, and that during this early interval of adjustment the zinc concentration in certain organs probably reaches a much higher level than is the case later when the excretory mechanism has adapted itself to its new requirements. With the exception of dog 5, all of our zinced animals have been killed after many weeks of zinc administration, so we have no confirmatory evidence of this explanation of the discrepancy between dog 5 and dogs 2 and 4, and are able to present it as a suggestion only.

SUMMARY AND CONCLUSIONS

Cats and dogs have been fed with daily doses of zinc oxide ranging from 175 to 1000 mgm. and for periods of time varying from three to fifty-three weeks. Clinical and laboratory studies made upon these animals include observations upon general health during the course of the experiment (weight determinations, appetite, nervous and digestive symptoms, condition of fur, etc.); hemoglobin and blood cell determinations; urine findings; gross and microscopic examination of tissues following autopsy; and zinc determinations in urine during the course of the experiment and in all organs and tissues after death.

As a result of these studies, we have never observed any significant clinical symptom nor obtained any significant laboratory evidence of damage resulting from the daily ingestion of zinc oxide over long periods of time. With the exception of fibrous change in the pancreases of three cats sub-

jected to doses of zinc very greatly in excess of any possible human exposure, histological examination following autopsy has shown in the organs of zinced cats and dogs no damage of any sort attributable to zinc.

The normal amount of zinc excreted daily in cat urine is shown to range between 0.06 and 0.43 mgm.; in dog urine, between 0.13 and 0.59 mgm.; in dog feces, between approximately 5.0 and 25.0 mgm.

Our studies upon zinc excretion in zinc-fed animals indicate that a small fraction of absorbed zinc leaves the body in the urine, but that the main bulk of it is excreted into the alimentary tract—some of it directly, some of it by the liver into the bile, and possibly some of it by the pancreas—and ultimately leaves the body in the feces. The amount of zinc in the urine of zinc-fed animals is markedly increased over the normal, though the total amount of zinc in the urine is but a small fraction of the total excreted. It is worthy of note that our animals excreted abnormal amounts of zinc through the kidneys for months without any histological evidence of kidney damage.

Figures are presented upon the normal zinc concentrations in the tissues and organs of cats and dogs, upon the zinc concentrations in the tissues and organs of zinced animals, and of zinced animals from which zinc administration had been discontinued for two weeks. These data indicate that the zinc concentrations of organs involved in the excretion of zinc (liver, gall bladder and bile, gastro-intestinal tract, and kidney) are increased as a result of zinc oxide ingestion, but that these abnormal concentrations fall rapidly to the normal level when zinc dosing is discontinued. Zinc concentrations in bone, bone marrow, and pancreas are also increased by zinc feeding—increases which fall towards the normal level on discontinuance of zinc. A possible excretory function of the pancreas is suggested in this connection.

The total zinc concentration of zinc-fed animals increases slightly with moderate zinc dosing and somewhat more markedly with excessive zinc dosing, but falls practically to a normal level within a short time (two weeks) after zinc dosing is discontinued.

A possible connection is suggested between the zinc content and the functional activity of the adrenals and of the sex glands.

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THE EFFECT OF ZINC ADMINISTRATION UPON REPRODUCTION AND GROWTH IN THE ALBINO RAT, TOGETHER WITH A DEMONSTRATION OF THE CONSTANT CONCENTRATION OF ZINC IN A GIVEN SPECIES, REGARDLESS OF AGE

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Received for publication September 17, 1926

THE EFFECT OF ZINC FEEDING UPON REPRODUCTION AND UPON THE GROWTH OF YOUNG RATS. A few years ago Bertrand and Vladesco (1920, 1921c) made the observation that the normal zinc concentration of various male reproductive organs and fluids in the sheep, bull, pig, horse, herring and man was often noticeably high compared with the concentration in the entire body. In the herring, a high concentration in the testes seemed to be correlated with the period of physiologic functioning. The results obtained in this laboratory during the past two years on rats, cats and dogs have indicated that the normal zinc concentration of both male and female reproductive organs is often very high. In the male cat and dog, the testes only were analyzed and were found to be rather low in zinc. In the rat, however, the seminal vesicles were included in the analysis and the zinc concentration was uniformly high compared with the concentration in the total animal. These findings gave added weight to the suggestion advanced by Bertrand and Vladesco that zinc may normally play a very important rôle in the phenomenon of reproduction. In this connection, we felt it of interest to determine whether or not zinc feeding would produce any variation from the normal in the content of the reproductive organs or in their physiological functioning.

Experimental procedures. The albino rat was chosen as being the most suitable animal in which to study growth and reproduction without unduly prolonging the experimental period. One year of a rat's life, as worked out by Donaldson (1924, p. 3) is approximately comparable to thirty years in the human cycle. White rats reproduce freely and often, the gestation period being only three weeks, and the number of young in a litter averaging about seven.

Our experimental parent rats were, for the most part, adult males and females which had received previously for twenty-nine weeks (from the time they were six weeks old), in the form of organic zinc salt solutions

(malate, acetate and citrate), daily doses of zinc ranging from 4.4 to 12.4 mgm. A few of the parent males, however, were white rats of different stock which had been on heavy suspensions of zinc oxide in gum acacia —34.4 mgm. of zinc daily—for twenty-nine weeks (from the time they were twenty-six weeks old).

Under the régime of zinc feeding, the organic zinc salt rats had grown in a normal manner and appeared perfectly healthy. The zinc oxide males, however, though their growth had been normal, had, unfortunately, been in contact with other rats which had so-called rat pneumonia. As it developed soon after the experiment was begun that the zinc oxide males were infected with this disease, they were eliminated from the experiment after they had served as sires in three matings, one of which proved sterile, and were replaced with one of the zinc malate sires and with another rat which had been on zinc acetate solution (2 mgm. of zinc daily) for forty-six weeks (from the time he was twelve weeks old), but which had been in isolated confined surroundings and on a slightly different diet. This zinc acetate rat was in good health, but was, like the zinc oxide sires, nearly twice as old as the female with which he was mated.

For controls, we mated rats which were of the same age and which had been maintained in the same surroundings for the same length of time and on the same diet as the zinc salt rats, but with water to drink instead of zinc salt solutions.

Throughout the experiment, the daily diet was as follows:

Stock mixture:	
Rolled oats	5 parts
Hominy	3 parts
Beef scraps	2½ parts
Milk powder	1½ parts
Salt	trace
Molasses horse feed (alfalfa, corn, oats and molasses)	
Dog biscuit	
Lettuce twice weekly	

Liquid, which was always measured in order to have a record of fluid consumption, was given either in the form of distilled water, a zinc salt solution, or a suspension of zinc oxide in 3 per cent gum acacia, according to the group to which the mated pair belonged. In the case of the zinc salt rats, the zinc dose was calculated from the fluid consumption. For the zinc oxide rats, a solution was made up weekly containing a known analyzed amount of zinc oxide and from this stock the cage burettes were filled as needed. At the end of the week all that was left of the stock solution was analyzed for zinc and subtraction of the two analyses gave the figure for the amount of zinc the pair of rats had ingested. This procedure was necessary, owing to the tendency of the zinc oxide to settle

TABLE 1

Results of fertility experiments with zinced rats and their controls

SERIES	PARENTS*			OFFSPRING		REMARKS
	Average daily dose of each pair of rats after mating (calculated as zinc)	Average daily dose of each rat before mating (calculated as zinc)†		September 28, 1925–January 2, 1926		
		Male	Female	Total number per female in number of litters	Deaths	
	mgm.	*mgm.*	*mgm.*			
D	Zinc acetate, 6.3	Zinc malate, 12.4	Zinc acetate, 4.4	6 in 2 litters	0	
E	Zinc malate, 16.5	Zinc malate, 12.4	Zinc acetate, 4.4	18 in 2 litters	0	8 embryos found at autopsy (1 litter)
F	Zinc malate, 11.1	Zinc malate, 12.4	Zinc citrate, 9.7	11 in 2 litters	1	
H	Zinc citrate, 10.2	Zinc oxide, 34.4	Zinc citrate, 9.7	15 in 3 litters	3	
L	Zinc acetate, 1.75	Zinc acetate, 2.0				
I	Zinc oxide, 6.5	Zinc oxide A, 34.4	Zinc citrate, 9.7	20 in 2 litters	19	Series I was sterile
J	Zinc oxide, 22.2	Zinc oxide B, 34.4				
M	Zinc oxide, 38.0	Zinc malate, 12.4				
Totals for five zinced females				70 in 11 litters	23	
Average number per liter				6.4		
Controls						
A	Water	Water	Water	14 in 3 litters	14	
B	Water	Water	Water	23 in 3 litters	12	
C	Water	Water	Water	19 in 2 litters	2	4 embryos found at autopsy (1 litter)
Totals for three control females				56 in 8 litters	28	
Average number per litter				7.0		

* All rats approximately 250 days old when mated, except the males in series H, L, I and J, which were approximately 410 days old.

† All rats were on zinc for 29 weeks previous to mating, except the male in series L (46 weeks).

out and thus to render inaccurate, dose figures obtained from fluid consumption.

The males were kept with the females throughout the experiment, so that second and third matings were dated from the time the first and second litters were born.

At the birth of a litter, two of the young were weighed and chloroformed for zinc analysis. The rest of the litter, regardless of number, was left with the mother for twenty-three days. The young rats were then weaned and distributed in other cages according to whatever zinc dose was allotted to them. In this manner, each pair of parent rats had two litters, each of which was allowed to grow to at least sixty days of age, in order to determine the effect of zinc on their early growth curves. In a few cases, three litters were born.

As the experiment carried to this point had not demonstrated any significant variation from the normal that could be attributed to zinc, we did not proceed with enlarging the numbers in the second generation or with breeding the second and third generations of rats, as had been our original plan, the work entailed far outweighing the value of the results to be anticipated.

Experimental results. Table 1 shows how the rats were mated; the periods of time during which they had been zinced previous to mating: their zinc doses during this experiment; the number of litters; the total number of offspring; and the number of young which died during the period they were with their parents. The figure for deaths among the young includes deaths from all causes—still-born births, young killed and eaten by the parents (a very common occurrence among normal rats (Evans and Bishop, 1923)), etc.—and is not at all an index of the health of the young at birth. No young rats died after weaning.

To the five females in the zinced group, seventy young were born (also eight unborn embryos found in one female at autopsy) during a 96-day period. There were eleven litters (the twelfth was embryonic) from fifteen matings. This is 15.6 offspring (including embryos) per mother, or 5.2 per mating. Thirty-two and nine-tenths per cent of the young born died or were killed by the parents.

In the control group, to the three mothers fifty-six young were born (also four embryos found) during the same period of time. There were eight litters (the ninth was embryonic) resulting from nine matings. This is 20.0 offspring, including embryos, per mother, or 6.6 per mating. Fifty per cent of the young born died or were killed by the parents.

The good health subsequent to weaning of the young in the various litters—zinced rats and water controls alike—as indicated in figures 1 and 2, which present the average growth curves of the various litters as compared with Donaldson's (1924, p. 178) average normal growth curves

for male and female albino rats. At the time of weaning, each litter was put on the same régime as the parents, i.e., litters from water parents were put on water, litters from zinc malate parents were put on zinc malate, etc. There was one exception to this procedure. Owing to lack of zinc oxide offspring, after weaning we put one of the second litters of zinc malate parents on zinc oxide.

The average curves given in figures 1 and 2 are made up of the following number of individual curves:

	Males	*Females*
Water rats	9 and	12
Zinc malate	13 and	4
Zinc citrate	3 and	5
Zinc acetate	4 and	1
Zinc oxide	3 and	3
Donaldson's normal rats	50 and	50

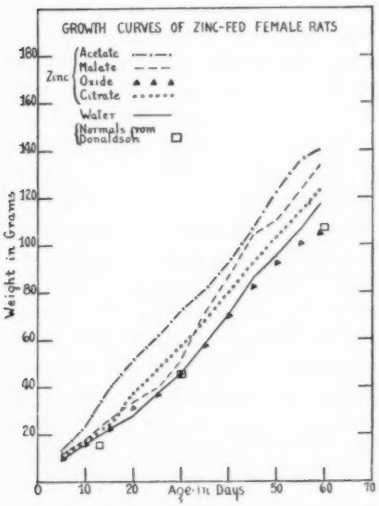

Fig. 1

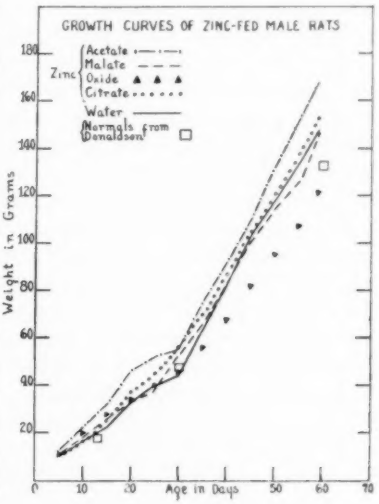

Fig. 2

Fig. 1. Growth curves of zinc-fed female rats plotted against the average growth curve of normal female rats, as determined by Donaldson.

Fig. 2. Growth curves of zinc-fed male rats plotted against the average growth curve of normal male rats, as determined by Donaldson.

Several of the second litters of offspring were transferred, at the end of the 60-day period, to another laboratory, where they were later used for breeding purposes. They reproduced in a perfectly normal manner, having large litters with no history of the death of any considerable number of the offspring.

At autopsy, the reproductive organs of the mated zinced male rats showed no difference in zinc concentration from that of unmated zinced males, and the reproductive organs of the zinc-fed rats showed no increase in zinc over those of the normals. Rat ovaries were not analyzed, since they are too small to give reliable analytical results.

Summarizing, we may say that the feeding of organic zinc salts or of zinc oxide in doses of from 2 to 38 mgm. of zinc daily to albino rats, not only for many weeks previous to mating, but during pregnancy and lactation as well, has no significant effect upon the health of the parents, upon fertility, nor upon the health and the early growth of the offspring. The offspring of zinced parents, weaned at twenty-three days of age and themselves put upon daily doses of zinc, grow at a normal rate and to a normal adult size.

The experiment has not thrown any light upon the cause of the high zinc concentration often found in reproductive organs, nor upon the possible rôle which zinc may play in the phenomenon of reproduction.

THE EFFECT OF ZINC FEEDING UPON THE TOTAL ZINC CONTENT OF RATS OF DIFFERENT AGES, TOGETHER WITH DATA UPON THE NORMAL ZINC CONTENT OF NEW-BORN, 60-DAY OLD AND ADULT RATS. In 1921, Bertrand and Vladesco (1921a) reported figures upon the zinc content of rabbits, guinea pigs, hens, mice and fish, raised under controlled conditions and ranging in age from three days to seven years. On the basis of figures obtained on the mouse, the hen, and the fish, *Idus Orfus*, these authors concluded that in general zinc is at a maximum in young life, being in all probability highest at birth; that it then diminishes gradually to a minimum level; and that it may rise again in old age. A second experiment by these same investigators (1921b) on rabbits a few hours to six weeks old, raised under controlled conditions but in a different laboratory and on a different diet from those in the first experiment, led to the conclusion that the total zinc content of the rabbit is at a maximum at birth, declines during lactation, and increases rapidly again when the animals are put on a regular mixed diet after being weaned. In our opinion, many more corroborative figures are needed in order to rule out normal variations which may possibly occur at any given age, even on a constant diet.

Giaya (1920) from analyses of viscera obtained at fourteen human autopsies on individuals who had given no history of having ingested zinc in excess of the normal amount in food, concluded that zinc content increases with age. The figures which Giaya presented support his assumption, but, in our opinion, the surroundings and diet of his subjects were too uncontrolled to warrant his results serving as a sound basis from which to draw such conclusions.

Lutz (1926) analyzing ten normal rats living on a constant diet, found approximately uniform concentrations of zinc (0.022 to 0.036 mgm. per

TABLE 2

Effect of zinc-feeding on zinc concentration of new-born, 60-day old and adult rats

AGE OF RAT	ZINC CONCENTRATION, MILLIGRAM OF ZINC PER GRAM OF TISSUE						REMARKS
	From water parents	From zinc malate parents	From zinc acetate parents	From zinc citrate parents	From zinc oxide parents		
New-born	0.030	0.038	0.033	0.033	0.034		
	0.030	0.039		0.042	0.043		
	0.030	0.040			0.052		
	0.032	0.042			0.054		
	0.032	0.048			0.042		
	0.040	0.051					
	0.040	0.052					
	0.040						
	0.041						
	0.050						
	0.051						
Average.....	0.038	0.044	0.033	0.039	0.045		
	On water	On zinc malate: 8.5 mgm. zinc daily	On zinc acetate: 5.0 mgm. zinc daily	On zinc citrate 16 mgm. zinc daily			
60 days	0.035	0.034	0.038	0.037			On zinc doses from the age of 3 weeks
	0.040	0.042	0.042	0.042			
	0.043	0.047		0.043			
		0.060					
Average.....	0.039	0.046	0.040	0.041			
	On water or 3 per cent gum acacia	On zinc malate: 13.8 mgm. zinc daily	On zinc acetate: 6.3 mgm. zinc daily	On zinc citrate: 10.2 mgm. zinc daily	On zinc oxide: 34.4 mgm. zinc daily	On zinc oxide: 0.5 to 24.0 mgm. zinc daily	
Adults 325± days		0.038	0.042	0.048			
		0.034	0.043	0.040			
		0.030					
			1 to 2 mgm. zinc daily				
450± days	0.038		0.043		0.035	0.042	Zinc salts rats on doses from age of 6 weeks: zinc oxide rats, from age of 26 weeks
	0.038		0.050		0.047	0.048	
	0.039				0.050	0.052	
	0.039				0.055	0.049	
	0.041				0.061	0.054	
	0.042					0.061	
						0.058	
Average.....	0.040	0.034	0.045	0.044	0.050	0.052	

gram) at all ages from approximately twenty-one days of age through an ascending series up to 160 days of age.

Fairhall[1] in this laboratory analyzed six approximately 500-day old rats, which had been for twenty-eight weeks under the strictly controlled régime of a normal zinc balance experiment, and found their total body concentration in zinc to be very uniform—viz., 0.032 to 0.036 mgm. per gram.

The control rat families in our fertility experiments provided us with excellent material for additional study upon the influence of age on zinc concentration in rats. We wished to see, furthermore, what effect, if any, zinc feeding might have on the total zinc concentration at different ages.

Experimental procedures. A few hours after a litter of rats was born, two members of the litter were chloroformed for analysis. A second group of rats was analyzed when sixty days old; and a third group in adult life.

The new-born animals were washed with dilute hydrochloric acid and distilled water, to remove any possible extraneous zinc that they might have picked up from their surroundings. All the other animals had the gastro-intestinal tract removed intact, slit up, and the contents washed out. The hides, and, in the case of the zinced animals, the mouths and throats were also thoroughly washed.

The methods of zinc analysis were those outlined by Fairhall (1926). Zinc, when under 2 mgm. in amount, was estimated by the ferrocyanide turbidimetric method; when over 2 mgm., by the ferrocyanide titration method.

Experimental results. The effect of zinc feeding upon the total zinc concentration of new-born, 60-day old, and fully grown rats is shown in table 2. This table also gives control figures upon the normal zinc concentration of rats of different ages. These latter figures for the total zinc concentration in normal rats are, on the whole, slightly higher than those of Fairhall and of Lutz—an observation which may possibly be accounted for by the fact that at the time of our experiment our stock foods were running higher in zinc than had been the case with theirs.

There is no indication from our figures of any correlation of the age of the animal with the total zinc concentration—the average zinc concentration (in milligrams per gram of tissue) of new-born, 60-day old and adult rats being, respectively, 0.038, 0.039 and 0.040 mgm. The individual figures in the three groups range from 0.030 to 0.051 mgm.[2]

[1] Unpublished data. Personal communication.

[2] Our opinion that new-born animals do not show a significantly higher zinc concentration than do adult animals of the same species is further confirmed by determinations of the zinc content of three new-born (placenta still attached) kittens, vigorous but of poor stock, found abandoned in the laboratory. We had no clue as to the mother's history except that she was a stray cat and had not been zinc-fed.

There seems also to be no significant increase in zinc concentration with zinc feeding, except possibly in the case of the adult zinc oxide rats, where both the average and the individual analyses are somewhat higher than those of normal rats of the same age. There are, however, a few cases where new-born and 60-day old rats have reached as high a level, but this was not maintained by the group as a whole. In terms of total zinc in the rat, a control adult with a zinc concentration of 0.040 and weighing 216 grams without intestinal contents had, for example, a total zinc content of 8.56 mgm. A zinc oxide adult of approximately the same weight, 218.6 grams (with a concentration of 0.056), had a total zinc content of 12.14 mgm. The total increase in zinc content during the long period of zinc feeding was, therefore, in this particular case 3.58 mgm., an amount not very significant in terms of storage.

SUMMARY AND CONCLUSIONS

The feeding of organic zinc salt solutions (acetate, citrate and malate) or of zinc oxide suspensions in doses of from 2 to 38 mgm. of zinc daily to albino rats, not only for many weeks previous to mating, but during pregnancy and lactation as well, has no significant effect upon the health of the parents, upon fertility, nor upon the health and early growth of the offspring.

The offspring of zinced parents, weaned at twenty-three days of age and themselves put upon daily doses of zinc, grow at a normal rate and to a normal adult size.

The average normal zinc concentration of albino rats does not vary significantly at different ages, the average total zinc concentrations of new-born, 60-day old, and of adult rats being, respectively, 0.038, 0.039 and 0.040 mgm. of zinc per gram of tissue. This constancy of zinc concentration, regardless of age, is suggestive evidence of a functional importance on the part of this metal.

With the exception of slight increases in total zinc concentration resulting from the feeding of heavy zinc oxide suspensions over long periods of time, the ingestion of zinc in the form of organic salt solutions or of oxide suspensions has no effect upon the total zinc concentration of the albino rat. That is to say, zinc compounds given by mouth to the rat do not usually result in storage of zinc in the animal in amounts above the normal.

Zinc analyses on these kittens gave the following figures for total zinc concentrations: 0.022, 0.023 and 0.030 mgm. per gram. The average zinc concentration of an adult cat according to Lutz (1926) is 0.033; according to our own observations, 0.031. The variation between these sets of figures is well within the normal variations at any one age.

If storage in excess of the normal quota does occur, the amounts stored are very small and have no effect upon the health of the animal.[3]

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[3] A detailed discussion of the absorption, excretion and storage of zinc, with a report of experiments upon the feeding of zinc to cats and dogs, may be found in a recent article by the authors (Drinker, Thompson and Marsh, 1927).

ARTIFICIAL HIBERNATION IN THE WOODCHUCK (ARCTOMYS MONAX)

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Received for publication September 18, 1926

The phenomenon of hibernation in the mammal is of interest, presenting, as it does, peculiar problems in heat regulation and metabolism. The transition from the homoiothermic to the poikilothermic state and the associated nutritional changes have been the subject of considerable research. Since much of the previous evidence seemed to indicate the existence of a close relationship between the mechanism of hibernation and the blood sugar level, the authors (1925) reported the results of experiments in which the combined effects of lowered body temperature and of insulin hypoglycemia were investigated. Inasmuch as animals that hibernate naturally were not obtainable at the time, dogs and cats were used. It was found that even in these animals a state simulating hibernation could be induced. The dogs and cats were anesthetized with amytal, cooled by immersion in a cold bath, and then given insulin. Two to four hours later, when the blood sugar concentration had fallen to 45 mgm. per cent (*circ.*), the animals ceased to shiver even though their body temperature was about 13°C. below the normal. The condition of the animals at this time was such as to suggest an analogy to the hibernating state particularly in the following respects: 1, the tendency of the animal to assume the temperature of its surroundings; 2, the absence of shivering and of spontaneous movements; 3, the loss of consciousness; 4, the profound reduction in the rate of metabolism; and 5, a prolonged state of hypoglycemia without convulsions. Since it appeared that the capacity for shivering was of prime importance as regards the recovery of normal temperature, and since this in turn had been found to depend upon the concentration of the blood sugar, it was concluded that insulin was related to the mechanism of hibernation. The results of similar experiments on an hibernating species are reported in the present communication.

American woodchucks (*Arctomys monax*) were used in this research. These animals were trapped during the month of May, and for a month

[1] Porter Fellow in Physiology.

preceding the experiments were kept in large wire cages, their food consisting for the most part of green vegetables, potatoes, and carrots. At the time of use the animals were all healthy and active. In the earlier experiments the method of procedure was the same as that described in our previous paper (1925). The protocol of one of these experiments follows:

Woodchuck 1. Weight 1.51 kgm.

10:51 Given 0.5 cc. of 10 per cent sodium amytal solution per kilogram intraperitoneally. Body temperature 38.7°C.
11:25 Immersed in a cold bath. Vigorous shivering
11:40 Body temperature 22.5°C. Removed from bath
12:00 Body temperature 20°C. Blood sugar 116 mgm. per cent
12:05 Given 3 units insulin per kilogram. Shivering vigorously
12:25 Body temperature 24°C. Animal wetted
12:50 Body temperature 23.3°C. Shivering vigorously
2:50 Body temperature 24°C. Shivering vigorously
3:15 Body temperature 25.1°C. Shivering vigorously
3:40 Blood sugar 56 mgm. per cent.
4:15 Body temperature 26°C. Animal given 3 units insulin per kilogram
4:25 Body temperature 27°C. Immersed in cold bath
4:31 Body temperature 22.6°C. Removed from bath
4:45 Body temperature 19.7°C. Animal still shivering
5:26 Blood sugar 67 mgm. per cent. Given 3 units insulin per kilogram
6:20 Body temperature 21.7°C. Shivering vigorously
7:30 Blood sugar 51 mgm. per cent. Given 5 units insulin per kilogram
8:30 Body temperature 25°C. Shivering vigorously
8:35 Given 5 units insulin per kilogram. Immersed in cold bath
8:45 Body temperature 23.5°C. Removed from bath. Shivering vigorously
11:00 Body temperature 25.5°C. Blood sugar 44 mgm. per cent. Shivering vigorously. Animal killed

In the above experiment shivering was not abolished at any time, and the temperature of the animal tended to rise whenever it was below normal. It was inferred that this was due to the fact that the blood sugar concentration did not at any time fall to a sufficiently low level to abolish shivering, even though large amounts of insulin had been injected. In order to produce a more profound hypoglycemia, another woodchuck was given insulin before immersion in the cold water; for, as has long been known, the emotional stimulation caused by the sudden exposure of an animal to cold produces a rise in the blood sugar concentration. The details of this experiment are given below:

Woodchuck 2. Weight 1.55 kgm.

June 21
10:45 Given 0.5 cc. of 10 per cent sodium amytal solution per kilogram
11:05 Given 0.1 cc. amytal per kilogram
11:30 Given 0.1 cc. amytal per kilogram
11:50 Body temperature 38°C. Blood sugar 161 mgm. per cent

12:00 Given 5 units of insulin per kilogram subcutaneously
4:00 Blood sugar 44 mgm. per cent
4:05 Given 5 units of insulin per kilogram subcutaneously
6:55 Animal completely relaxed
7:10 Body temperature 37°C. Blood sugar 36 mgm. per cent
8:18 Body temperature 37°C. Feeble tonic convulsion
8:30 Immersed in cold bath. No shivering
8:40 Slight shivering. Removed from bath. Blood sugar 97 mgm. per cent
8:45 Immersed in bath
9:07 Removed from bath. Slight shivering. Body temperature 22°C.
9:28 Body temperature 21.5°C.
10:55 Body temperature 18°C. Shivering vigorously
11:25 Blood sugar 70 mgm. per cent. Given 5 units of insulin per kilogram subcutaneously

June 22

12:15 Body temperature 26°C. Shivering vigorously. Immersed in bath
12:27 Body temperature 20.8°C. Shivering vigorously. Removed from bath
12:50 Body temperature 20.3°C. Shivering vigorously
1:40 Body temperature 26.5°C. Shivering vigorously. Given 10 units of insulin per kilogram
9:50 Animal in a state of profound torpidity, lying partly turned on its back with limbs outstretched. Body temperature 24°C. Temperature of room 23°C.
10:54 Body temperature 24°C. Blood sugar 19 mgm. per cent
2:12 Body temperature 25°C. Temperature of room 23.5°C.
4:36 Body temperature 25.5°C. Temperature of room 23.4°C.
9:30 Body temperature 25.7°C. Temperature of room 23.5°C.

June 23

9:00 Body temperature 30°C. Temperature of room 24°C. Blood sugar 59 mgm. per cent. Animal semi-torpid and capable of slow movement, if stimulated. Wetted with cold water. No shivering
9:10 Given 5 units of insulin per kilogram
11:15 Body temperature 21.5°C.
5:21 Body temperature 22.5°C. Blood sugar 60 mgm. per cent. Feeble movements and slight shivering. Animal died during removal of sample

The blood sugar concentration of this woodchuck fell to 36 mgm. per cent 7 hours and 10 minutes after the first administration of 5 units of insulin per kilogram. When the animal was immersed in a cold bath, no shivering was observed until 10 minutes had elapsed. At the moment when shivering was first noticed, the blood sugar had risen to 97 mgm. per cent. This indicates that hypoglycemia abolishes shivering in the woodchuck, but that immersion of an hypoglycemic woodchuck in cold water causes a marked increase in the blood sugar concentration, and this is followed by shivering. As previously pointed out by the authors (1926a) the domestic fowl behaves similarly.

Once the body temperature of the woodchuck had been lowered, and profound hypoglycemia induced by means of repeated injections of insulin, the animal passed into a state of artificial hibernation. Before assuming this state of torpidity, the woodchuck was shivering vigorously,

and its temperature rose from 20.3°C. to 26.5°C. in 50 minutes. Several hours later, when the blood sugar had fallen to 19 mgm. per cent and shivering had disappeared, the animal's temperature fell to 24°C. and remained at approximately this level for 12 hours, the surrounding temperature varying during this period from 23°C. to 23.5°C. The following morning (June 23) the temperature of the animal had risen to 30°C., and the woodchuck was in a semi-torpid condition; *i.e.*, stimulation evoked slow body movements. It was found that the blood sugar concentration had increased to 59 mgm. per cent. The woodchuck was wetted with cold water, and given an additional 5 units of insulin per kilogram. Its temperature fell rapidly to 21.5°C., and the animal once more became completely torpid. However, the tendency of the blood sugar level to rise was still marked; for, in spite of the insulin administration, the blood contained 60 mgm. per cent of sugar at 5:21 p.m. At this time the animal began to shiver and move about, although its temperature had risen only 1°C.

From the above it became apparent that it would be necessary to administer insulin at intervals in order to keep these animals in a state of torpidity for a considerable period of time. In order to simulate more closely natural conditions, it was decided to give insulin to woodchucks and then to place them in a cool chamber. This procedure made the use of amytal unnecessary, and, in addition, eliminated the excessive stimulation caused by immersion in cold water. It was also possible to keep the animals completely dry, so that evaporation of water would not be a factor in lowering their body temperature. The cooling chamber was the same as that used by Tait and Britton (1923) in a series of experiments on the artificially cooled woodchuck. It was a large, felt-covered, double-walled chamber with a glass window through which the animal could be observed. The space between the walls was filled with ice. The animals were placed in a wire cage within the chamber, to prevent them from coming into contact with the cold walls.

As a control experiment, a woodchuck without insulin was placed in the cooling chamber for a period of 8 hours. It was able to maintain a body temperature of 37.3°C. without shivering or moving about to any considerable extent. The temperature of the chamber varied from 6°C. to 13°C. The animal was then given 30 units of insulin per kilogram. The following morning this woodchuck was partially torpid and a few hours later was completely dormant. Its temperature at this time was 28°C.

Another woodchuck was given insulin (25 units per kilogram), at 3 p.m. on June 24. At 6 p.m. the animal was inactive, and at 10:15 p.m. semi-torpid. On the morning of June 25 the animal was in a state of profound

torpor, its temperature having fallen to 12.1°C. The temperature of the chamber was 11°C. At 8:30 that evening the temperature of the chamber had risen to 16.5°C. and the temperature of the animal was 17.8°C. The woodchuck was given a second injection of insulin. The next morning (June 26) it was still completely torpid in spite of the fact that its temperature had risen to 28°C. At this time the temperature within the chamber was 22.5°C. The chamber was repacked with ice. At 10:05 p.m. the temperature within the chamber had fallen to 11°C. and that of the animal to 10°C. The woodchuck was completely dormant and even

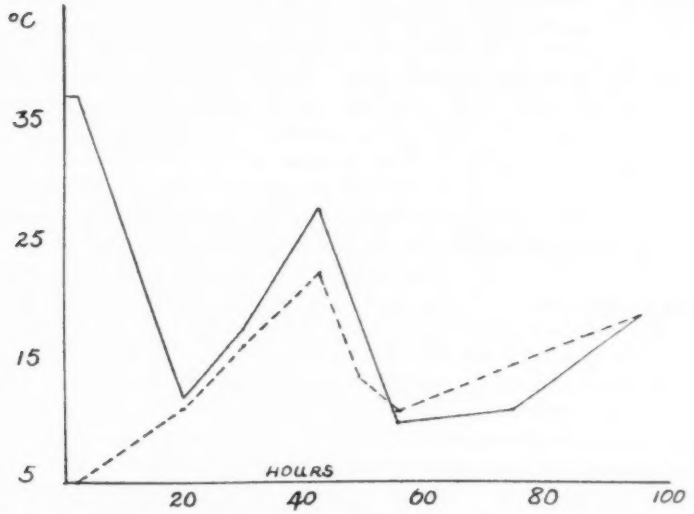

Fig. 1. Curves showing the relation of the temperature of an hypoglycemic woodchuck to that of its environment. Continuous line—temperature of animal. Broken line—temperature of environment.

the corneal reflex was absent. The next evening (June 27) the animal's temperature was 11.3°C. that of the chamber being 15°C. The animal received a third injection of insulin (10 units per kilogram). On the afternoon of June 28 this woodchuck was still asleep. The temperature of the animal was the same as that of the chamber—19°C. The relation of the temperature of this animal to that of its environment is shown in figure 1. At 3:18 p.m. this animal received 2 cc. of adrenalin (1:1000 solution) subcutaneously, in order to increase the blood sugar concentration and thus cause the animal to shiver (the authors, 1926b). Fifteen minutes after the injection there was a marked increase in the rate and

depth of respiration. Six minutes later the woodchuck began to shiver and to move about. Unfortunately the animal died during an attempt to obtain a sample of blood from the heart. A similar experiment was carried out on an amytalized woodchuck. This animal remained dormant for 2½ days at the end of which time it died.

Tait and Britton (1923) found that woodchucks recovered their normal temperature spontaneously and promptly after having been cooled artificially to temperatures varying between 12°C. and 3°C. As has been described above, a woodchuck with hypoglycemia becomes poikilothermic; its temperature closely parallels that of its surroundings. The change from the homoiothermic to the poikilothermic condition is undoubtedly a consequence of the loss of the capacity for shivering and the ability to carry out spontaneous movements. It has been shown by Morgulis (1924) that the increase in metabolic rate produced by the exposure of an animal to cold is due entirely to increased muscular activity.

The degree of torpidity observed in these animals varies from time to time, and seems to be influenced by outside stimulation. Often when subjected to continued manipulation a torpid woodchuck begins to execute slow feeble movements; as soon as it is left undisturbed, it becomes completely torpid again. The animals lie inert and are usually partly turned on their back. During some periods there is little or no muscular tone, the knee-jerk and corneal reflexes are absent, and the animals do not respond to painful stimulation. At other times there is considerable muscular tone, and the knee-jerk and corneal reflexes are present. During profound torpor the respirations are very slow and barely perceptible. Occasionally a long deep respiration occurs. Biot breathing was observed in some instances. Immediately before becoming dormant the animals passed urine and feces. During torpidity small amounts of urine were occasionally voided. Whereas insulin convulsions may be produced readily in woodchucks at normal body temperature, no convulsions were observed in these animals when their body temperature had been lowered. Hence, lowered body temperature inhibits insulin convulsions in woodchucks as it does in dogs and cats.

The authors (1925) have shown that administration of glucose to a cooled hypoglycemic dog or cat causes the shivering reflex to reappear, and as a result the animal regains its normal condition. Two grams per kilogram of glucose in 20 per cent solution were injected into a woodchuck after artificial hibernation had been induced. Shivering began 4 minutes later and became vigorous within 8 minutes. Sixteen minutes after receiving the sugar, the animal was actively moving about and interested in its surroundings. Within 4 hours its temperature had risen to 36.5°C., and the woodchuck was entirely normal.

SUMMARY

When a woodchuck is given an amount of insulin sufficient to produce profound hypoglycemia, it loses its power of temperature control. If it then be placed in an environment even moderately cool, it passes into a state of artificial hibernation. The characteristics presented by an animal in this condition are: 1, the woodchuck becomes poikilothermic; 2, it is unable to shiver or execute spontaneous movements; 3, consciousnes is lost, and the animal is insensible to painful stimulation; 4, the convulsions associated with insulin hypoglycemia do not occur; 5, the metabolic rate is greatly decreased. The state of torpor thus induced can be prolonged by the administration of insulin at intervals. The injection of glucose terminates this condition. Shivering begins almost immediately, the temperature rises at a rapid, though variable, rate, and the animal returns to normal.

Our thanks are due to Prof. John Tait whose kind coöperation and previous experiments greatly facilitated this research.

We also wish to thank Dr. G. J. Cassidy for the valuable assistance that he has given us in the early part of this work.

The expenses of this research were defrayed in part by a grant from the James Cooper Fund of McGill University for Research in Experimental Medicine.

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THE EFFECT ON THE URINARY OUTPUT OF WATER, CHLORIDE, INORGANIC PHOSPHATE, UREA AND AMMONIA OF VARYING THE SALT AND ACID CONTENT OF WATER DRUNK

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Received for publication September 20, 1926

In the laboratory course in physiology given at this school an experiment on urine secretion was performed by the students in the fall of 1925. The object of the experiment, aside from purposes of instruction, was to collect data on the effect on the urinary output of water, chloride, inorganic phosphate, urea and ammonia of varying the salt and acid content of water drunk. The experiment was carried out as follows.

The laboratory work in the course is done by students working in pairs; there were thirty-six pairs of students. For this experiment these thirty-six pairs were divided into twelve groups of three pairs each. One member of one pair in each group acted as subject, his partner measured the volumes and specific gravities of the samples and made the phosphate determinations. Another pair made the urea and ammonia determinations and the third pair determined the chlorides. The collection of samples was as follows: A normal subject, with protein-free urine, took nothing by mouth after 11:00 p.m. of the evening before the experiment. At exactly 8:00 a.m. he emptied his bladder, discarding the urine, and drank 500 cc. of distilled water; the bladder was then emptied at 9, 10, 11 and 12 o'clock. On the afternoon of the same day the analyses were made. On each experimental day for each group analyses of the various constituents were thus made on four consecutive hourly samples. On the second experimental day the procedure was exactly as outlined above except that the subject drank 500 cc. of 0.3 per cent NaCl instead of distilled water, on the third day 500 cc. of 0.6 per cent NaCl and on the fourth day 500 cc. of 0.9 per cent NaCl. On the fifth, sixth, seventh and eighth experimental days the subject drank 500 cc. of $N/100$ HCl solution containing 0, 0.3 per cent, 0.6 per cent and 0.9 per cent NaCl, respectively. The experimental days for any one group were scattered throughout the fall trimester. The complete data for one group thus give the specific gravity, water, chloride, phosphate, urea and ammonia output for four consecutive hourly periods on each of eight days, different solu-

tions being drunk each day. There are twelve such sets of data, one for each group of three pairs. During the mornings of collection of samples the subjects performed their ordinary school work. Chlorides were determined by the method of Austin and Van Slyke, inorganic phosphates by Briggs' method, urea by urease action and direct Nesslerization and ammonia by the permutit method. On the first experimental day for each group its members were carefully instructed in regard to the various chemical procedures; the importance of conscientiously observing the rules regarding the food and water intake and of careful collection of samples was emphasized with the subject. The chemical work of every group of every day was under practically constant supervision; numerous duplicate determinations were carried out to check the students' work, and probably more than half of the students' figures were checked by recalculation from their titration or colorimeter readings. The students engaged in this work had all had the course in biological chemistry. There is every reason to believe that the great majority of the figures obtained are reasonably nearly correct.

Each of the twelve groups worked with four urine samples on each of eight days. On each of these samples six procedures were carried out, measurement of volume and of specific gravity, determination of chloride, phosphate, urea and ammonia. Each group thus carried out 192 procedures; the data for the twelve groups include 2,304 figures, 768 measurements of volume and specific gravity and 1,536 chemical analyses on 384 urine samples. It is evident that this mass of data cannot be presented in this paper. To conserve space and at the same time have all the data represented, tables of averages have been constructed. Thus table 1 represents the average of the twelve groups for the day on which distilled water was drunk, table 2 for 0.3 per cent NaCl, etc. All subjects were normal males, average age 22 years, average weight 68.2 kilos.

Water output. It will be noted that with either neutral or acid water the urine output of the first four hours falls off progressively as the salt content approaches that of an isotonic solution. This observation was reported by Starkenstein (1924). In no case does the urine output of the first four hours following the drinking equal the amount of water or solution drunk. It will also be noted that the hourly rate of elimination falls off much less rapidly as the salt content increases; with the isotonic solution the hourly output is almost a constant. If the individual tables of the twelve subjects (not published in this paper) are examined, however, numerous exceptions are seen to all of the above statements which are true of the average tables. Thus subject T.A. excreted 955 cc. urine in the four hours following the drinking of 500 cc. distilled water, 670 cc. after 0.3 per cent NaCl, 530 cc. after 0.6 per cent NaCl and 394 cc. after 0.9 per cent NaCl; in every case except with isotonic salt solu-

TABLE 1

500 cc. distilled water at 8:00 a.m.

SAMPLE	TIME	URINE PER HOUR	SPECIFIC GRAVITY	NaCl PER 100 CC.	NaCl PER HOUR	P PER 100 CC.	P PER HOUR	UREA N PER 100 CC.	UREA N PER HOUR	AMMONIA N PER 100 CC.	AMMONIA N PER HOUR
		cc.		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	8– 9	130	1.013	456	593	19.1	24.8	375	488	15.9	20.6
2	9–10	159	1.013	512	814	9.2	14.6	312	495	9.2	14.7
3	10–11	73	1.019	822	600	16.9	12.3	466	340	16.0	11.7
4	11–12	49	1.023	1089	534	32.7	16.0	706	346	19.8	9.7
Total for 4 hours.....		411			2541		67.7		1669		56.7

TABLE 2

500 cc. 0.3 per cent NaCl at 8:00 a.m.

SAMPLE	TIME	URINE PER HOUR	SPECIFIC GRAVITY	NaCl PER 100 CC.	NaCl PER HOUR	P PER 100 CC.	P PER HOUR	UREA N PER 100 CC.	UREA N PER HOUR	AMMONIA N PER 100 CC.	AMMONIA N PER HOUR
		cc.		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	8– 9	143	1.015	530	757	13.5	19.3	307	438	11.5	16.5
2	9–10	96	1.017	680	652	12.7	12.2	359	345	14.1	13.5
3	10–11	48	1.021	1180	566	22.3	10.7	544	261	20.9	10.0
4	11–12	35	1.024	1316	461	36.6	12.8	623	218	26.3	9.2
Total for 4 hours.....		322			2436		55.0		1262		49.2

TABLE 3

500 cc. 0.6 per cent NaCl at 8:00 a.m.

SAMPLE	TIME	URINE PER HOUR	SPECIFIC GRAVITY	NaCl PER 100 CC.	NaCl PER HOUR	P PER 100 CC.	P PER HOUR	UREA N PER 100 CC.	UREA N PER HOUR	AMMONIA N PER 100 CC.	AMMONIA N PER HOUR
		cc.		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	8– 9	100	1.021	533	533	16.7	16.7	397	397	18.7	18.7
3	9–10	51	1.023	971	495	24.3	12.4	672	343	33.7	17.2
3	10–11	33	1.025	1373	453	22.7	7.5	945	312	42.4	14.0
4	11–12	33	1.026	1255	414	32.8	10.8	898	296	42.4	14.0
Total for 4 hours.....		227			1895		47.4		1348		63 9

TABLE 4

500 cc. 0.9 per cent NaCl at 8:00 a.m.

SAMPLE	TIME	URINE PER HOUR	SPECIFIC GRAVITY	NaCl PER 100 CC.	NaCl PER HOUR	P PER 100 CC.	P PER HOUR	UREA N PER 100 CC.	UREA N PER HOUR	AMMONIA N PER 100 CC.	AMMONIA N PER HOUR
		cc.		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	8–9	60	1 024	1142	685	35.8	21.5	702	421	23.7	14 2
2	9–10	62	1.023	1347	836	37.1	23.0	650	403	24.8	15.4
3	10–11	45	1.024	1485	668	31.4	14.1	693	312	27.1	12 2
4	11–12	42	1.025	1533	644	33.6	14.1	664	279	21.4	9.0
Total for 4 hours.....		209			2833		72.7		1415		50 8

TABLE 5

500 cc. N/100 HCl at 8:00 a.m.

SAMPLE	TIME	URINE PER HOUR	SPECIFIC GRAVITY	NaCl PER 100 CC.	NaCl PER HOUR	P PER 100 CC.	P PER HOUR	UREA N PER 100 CC.	UREA N PER HOUR	AMMONIA N PER 100 CC.	AMMONIA N PER HOUR
		cc.		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	8– 9	188	1.013	389	731	15.0	28.1	274	515	12.7	23.9
2	9–10	171	1.012	384	656	12.7	21.7	302	517	12.7	21.7
3	10–11	71	1.019	710	504	27.3	19.4	442	314	20.9	14.8
4	11–12	52	1.023	881	458	55.0	28.6	548	285	22.7	11.8
Total for 4 hours.....		482			2349		97.8		1531		72.2

TABLE 6

500 cc. 0.8 per cent NaCl N/100 HCl at 8:00 a.m.

SAMPLE	TIME	URINE PER HOUR	SPECIFIC GRAVITY	NaCl PER 100 CC.	NaCl PER HOUR	P PER 100 CC.	P PER HOUR	UREA N PER 100 CC.	UREA N PER HOUR	AMMONIA N PER 100 CC.	AMMONIA N PER HOUR
		cc.		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	8–9	105	1.017	586	616	28.4	29.8	498	523	23.1	24 3
2	9–10	90	1 019	760	684	25.6	23.0	492	443	25.6	23.0
3	10–11	58	1.022	997	578	36.3	21.1	622	361	34.5	20.0
4	11–12	53	1.022	1116	592	41.9	22.2	636	337	34.3	18.2
Total for 4 hours.....		306			2470		96.1		1664		85.5

TABLE 7

500 cc. 0.6 NaCl in N/100 HCl

SAMPLE	TIME	URINE PER HOUR	SPECIFIC GRAVITY	NaCl PER 100 CC.	NaCl PER HOUR	P PER 100 CC.	P PER HOUR	UREA N PER 100 CC.	UREA N PER HOUR	AMMONIA N PER 100 CC.	AMMONIA N PER HOUR
		cc.		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	8– 9	92	1.016	654	601	23.5	21.6	467	430	36.2	33.3
2	9–10	73	1.018	906	661	19.3	14.1	575	419	29.2	21.3
3	10–11	63	1.020	940	592	24.5	15.4	562	354	28.1	17.7
4	11–12	44	1.023	1100	484	34.6	15.2	636	280	34.8	15.3
Total for 4 hours.....		272			2338		66.3		1483		87.6

TABLE 8

500 cc. 0.9 per cent NaCl in N/100 HCl

SAMPLE	TIME	URINE PER HOUR	SPECIFIC GRAVITY	NaCl PER 100 CC.	NaCl PER HOUR	P PER 100 CC.	P PER HOUR	UREA N PER 100 CC.	UREA N PER HOUR	AMMONIA N PER 100 CC.	AMMONIA N PER HOUR
		cc.		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	8– 9	55	1.022	996	548	44.4	24.4	727	400	39.5	21.7
2	9–10	66	1.022	1020	673	34.2	22.6	598	395	31.2	20.6
3	10–11	53	1.023	1129	599	36.8	19.5	717	380	37.3	19.8
4	11–12	45	1.024	1345	605	43.8	19.7	729	328	36.5	16.4
Total for 4 hours.....		219			2425		86.2		1503		78.5

tion the urine output exceeded the water intake. Subject B. R. had his highest output with 0.6 per cent NaCl, H. O., R. O. and A. N. with 0.3 per cent NaCl; the highest output for the other eight subjects was with distilled water.

On comparing the urine outputs after neutral solutions with the outputs after the acid solutions of the same salt content it is seen that the tables of averages show a greater output with the acid solutions in every case but that of 0.3 per cent NaCl. The slightly higher average output with neutral solution here is due to the extremely large output of urine after neutral 0.3 per cent NaCl by subject H. O., 895 cc., whereas he passed only 244 cc. after the acid 0.3 per cent NaCl. On turning to H. O.'s tables with the idea of finding a possible explanation for this great output after neutral 0.3 per cent NaCl solution the note was found that the first hour after drinking the solution was spent in the recumbent position. The solution was drunk at 8:00 a.m. and the subject stayed in

bed after drinking. The output during this hour was 402 cc. The first half of the second hour was also spent in bed, the output for the second hour being 253 cc. The fact that he was in bed during the first hour and a half of this period has been confirmed by personal communication with the subject. No part of the period after drinking the acid 0.3 per cent NaCl was spent in the recumbent position and the output for the four hours was 244 cc. This finding of an increased urine output in recumbency has been considered in detail in a recent paper by White, Rosen, Fischer and Wood (1926). It is quite possible that uncontrolled variations of posture, which were not noted on the tables by the other subjects, have played a part in the rather numerous individual exceptions to the average results. A diuresis on drinking acids or solutions of acid salts, over and above that produced by the same volume of neutral solutions, has been noted by numerous previous observers. The increases on acid drinking seen in these tables are not so pronounced as some previously reported instances because the acid intake in this work was relatively small. The mechanism of this action is still obscure. An explanation based on the shift of the reaction of the body proteins toward their isoelectric point hardly seems valid in the case of such a small acid intake as was employed in these experiments, although it must be admitted that the diuretic effect was not great and might be accounted for by an apparently insignificant change of reaction.

Chloride output. There is obviously no close relation between the chloride intake and the chloride output of the first four hours. The chloride output of the first four hours after drinking 500 cc. of distilled water is greater (2,541 mgm.) than after drinking 500 cc. of 0.3 per cent NaCl solution (2,436 mgm.) or 0.6 per cent NaCl solution (1,895 mgm.). With 0.9 per cent NaCl however, the chloride output is 2,833 mgm. On drinking the acid solutions the chloride output is relatively unchanged, regardless of the chloride intake. The average chloride output for the four periods is practically the same with neutral or acid solutions. It is interesting to note that variations in urine output brought about by varying the salt content of the ingested water are not accompanied by significant variations in the rate of chloride output, while variations in urine output brought about by changes in posture are accompanied by marked variations in rate of chloride output (White, Rosen, Fischer and Wood, 1926).

Inorganic phosphate output. On drinking neutral solutions the phosphate output for the four hours following the drinking falls off progressively as the chloride content of the solution drunk increases from 0 to 0.6 per cent NaCl. With 0.9 per cent NaCl however, the phosphate output rises even higher than that after drinking distilled water. Also, with acid solutions the phosphate output falls off as the chloride content of the

solution drunk rises from 0 to 0.6 per cent NaCl, increasing with 0.9 per cent NaCl but not to the height reached after the NaCl-free acid water. Whether or not these facts are due to anything more significant than mere coincidence we cannot say; the individual tables show several exceptions to this average behavior. A progressive decrease in the rate of phosphate output from hour to hour is seen in most of the tables; this observation has been reported by previous investigators.

On comparing the phosphate output after acid solutions with that after neutral solutions a significant increase is seen. Every acid solution period shows a clearly greater phosphate output than the period after the neutral solution of corresponding NaCl content. Gamble, Blackfan and Hamilton (1925) found no significant change in phosphate output determined on 24 hour urine specimens after much larger intakes of acid (500 to 1,000 cc. daily of $\frac{N}{10}$ solutions of acid-producing salts) than were employed in our experiments. Apparently the increased phosphate output after acid seen in our experiments is only temporary and is compensated in a 24 hour sample by a later decrease; the probability of this is strengthened by the fact that the output from hour to hour is progressively diminishing during the first four hours in the majority of cases. One might expect any increase in the acid-excreting rôle of phosphate after such small acid intakes as obtained in our experiments to consist merely in an increase in the proportion of acid to alkaline phosphates. The finding of an even temporarily increased amount of phosphate excreted was somewhat surprising. It may be noted that Haldane, Hill and Luck (1923), contrary to Gamble, Blackfan and Hamilton, found an increased 24 hour output of phosphate after drinking large amounts of $CaCl_2$ solution.

Urea output. The rate of urea excretion bears no consistent relation in these experiments to water output or to chloride or acid content of solutions drunk. There is a progressive falling off in the output from hour to hour in practically every case.

Ammonia output. The rate of ammonia excretion is apparently independent, in these experiments, of the water output and chloride content of solutions drunk. It is, as would be expected, significantly increased in the "acid periods" as compared with the "neutral periods." There are on the average 25 mgm. more of ammonia N excreted in a four-hour "acid period" than in a "neutral period;" this corresponds to 178 cc. $N/100$ acid. There are 20.2 mgm. more of P excreted in an "acid period" than in a "neutral period;" this corresponds to 45 cc. $N/100$ acid.[1] It is

[1] It was not practicable to determine urinary pH in this work, but assuming an average urine pH of 5.9 after drinking acid solutions, a transfer of 20 mgm. P from the plasma at a pH of 7.4 to the urine at a pH of 5.9 involves the change from a state of Na_2HPO_4 to a state of NaH_2PO_4 of 14 mgm. P. Because at pH 7.4 two-tenths of the

thus seen that of 500 cc. $N/100$ acid taken in, 223 cc. are excreted in the first four hours buffered with the extra ammonia and phosphate excreted.

SUMMARY

The effect on the urinary output of water, chloride, inorganic phosphate, urea and ammonia of varying the NaCl and acid content of 500 cc. ingested water was determined.

The output of water in the first four hours following the drinking is diminished by increasing the NaCl content up to 0.9 per cent. The output of urea and ammonia is independent of the NaCl content of the ingested water, the output of phosphate falls off as the chloride content rises from 0 to 0.6 per cent, then rises with 0.9 per cent NaCl. The chloride output is in general independent of the chloride intake.

The output of water, phosphate and ammonia is increased by taking acid, the output of chloride and urea is unaffected by the acid.

These findings are those of the tables of averages; numerous exceptions to the above statements are found in the tables of the individual subjects.

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phosphate is the acid and eight-tenths the alkaline, i.e., 4 mgm. of the 20 mgm. P exist as NaH_2PO_4 and 16 mgm. as Na_2HPO_4, while at pH 5.9 nine-tenths are acid and one-tenth alkaline, i.e., of 20 mgm. P, 18 mgm. exist as NaH_2PO_4 and 2 mgm. as Na_2HPO4. This represents the conversion of 14 mgm. P from a state of Na_2HPO_4 to NaH_2PO_4. Since one hydrogen ion is involved in the change of each phosphate molecule, it would require 1 cc. of $\frac{N}{1}$ or 100 cc. of $\frac{N}{100}$ acid to change 31 mgm. P from an alkaline to an acid phosphate. To change 14 mgm. P requires $\frac{14}{31} \times 100$ or 45 cc. of $\frac{N}{100}$ acid.

THE UNITY OR MULTIPLICITY OF THE AUTACOIDS OF THE POSTERIOR LOBE OF THE PITUITARY GLAND[1]

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Received for publication September 21, 1926

The important and valuable physiological properties of pituitary extracts have led to a number of attempts to obtain the autacoids of the lobe in a pure form. Up to the present time, these attempts have failed and the chemical nature of these substances remains obscure. Recently, however, considerable progress has been made in their purification by Dudley and by Abel and his collaborators, and the results obtained by these workers have led to a controversy on the question of the unity or multiplicity of the autacoids responsible for the pressor, renal and oxytocic activities of the extract. A brief resumé of these opposing views is presented below.

Dudley (1) found that normal butyl alcohol extracted from an aqueous extract of the lobe nearly all of the oxytocic activity but left behind in the aqueous layer 50 per cent of the pressor activity. Dudley took this as evidence that the pressor and oxytocic activities of the extract were due to separate chemical individuals.

The accuracy of Dudley's results has been questioned by Hogben and Schlapp (2) who pointed out that Dudley's extracts contained the ethyl alcohol soluble depressor substance, which was first demonstrated by Schafer and Vincent (3), and that the presence of this depressor substance interferes materially with the accuracy of pressor assays. Later Hogben, Schlapp and MacDonald (4) proposed an improved technique for pressor standardization in which the depressor substance is first removed by ethyl alcohol extraction and the assay made on a pithed cat. This method, under favorable conditions, will yield an assay accurate to within 10 per cent or less.

During the progress of the experiments reported in the paper Schlapp (5), using the technique of Hogben, Schlapp and MacDonald, repeated Dudley's work and confirmed his findings.

Knauss (6), working with pithed cats, found that the ratio of the minimal doses of the extract, acting upon the uterus in situ and the blood pres-

[1] Presented in partial fulfilment of the requirements for the degree of Master of Science, McGill University.

sure, varies according to whether the injection is made intravenously or intra-arterially. This author concludes therefrom that there are separate pressor and oxytocic constituents and that the pressor substance is destroyed by passing through the arterioles.

There is some evidence that the three activities are not present in constant proportions in all parts of the lobe. Herring (7) found that extracts of the pars nervosa have a higher oxytocic than pressor activity as compared with extracts of the pars intermedia. Recently Hogben and DeBeer (8) reached the same conclusions.

The belief that one constituent is responsible for the pressor, oxytocic, and renal activities of the lobe rests chiefly upon the work of Abel and his collaborators. These authors (9), during the course of chemical manipulations designed to isolate the active constituents of the lobe, found that the pressor, oxytocic and renal activities of their preparations increased pari passu as the purification proceeded. Their most highly purified substances were in the form of "tartrates." One of these "tartrates" having an oxytocic titer of 160 times histamine acid phosphate was found, when assayed against a simple aqueous extract of the lobe, to possess the three activities standing in the same relationships to each other as in the simple aqueous extract. The authors present no data in support of this point.

Abel and his collaborators, in view of their results, are led to believe that the three activities are due to one constituent. "The probabilities" they say, "are entirely against the concomitant and progressive purification of three or more separate substances by the chemical methods we have described."

Although no data are given for the assay of the "tartrate" having a titer of 160 times histamine acid phosphate, the authors present data in the same paper showing the effect of a more highly purified "tartrate" having a titer of 500 times histamine acid phosphate on the blood pressure and urine excretion. A study of these data reveals the fact that the three activities were not preserved in their original proportions. On the contrary, a great loss of the pressor and diuretic activities appears to have taken place. In figure viii of (9) 0.2 mgm. of a tartrate having an oxytocic titer of 500 times histamine acid phosphate is shown to cause a slight rise of blood pressure in an anesthetized cat and 0.05 mgm. is shown to produce a submaximal diuretic effect in an anesthetized rabbit. These doses had the oxytocic activity of 100 mgm. and 25 mgm. of histamine acid phosphate, respectively, and were the oxytocic equivalent of 100 cc. and 25 cc. of the liquor hypophysis of the U.S.P. IX. Since $\frac{1}{10}$ to $\frac{1}{100}$ cc. of liquor hypophysis is sufficient to raise the blood pressure of a cat or to promote diuresis in a rabbit, it is evident that these activities had been almost completely destroyed or eliminated in the process of purification.

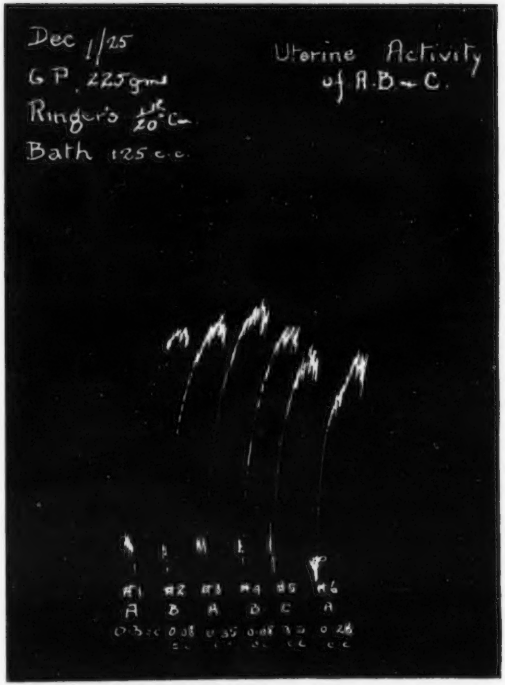

Fig. 1. Shows the relative oxytocic activities of the three fractions. The oxytocic activity of 1.0 cc. of A is equivalent to that of 0.263 cc. of B, 10.7 cc. of C.

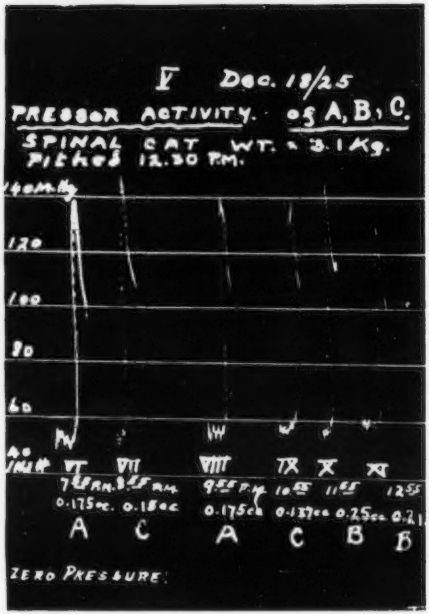
F Dec. 18/25
PRESSOR ACTIVITY. of A, B, C.
SPINAL CAT WT. = 3.1 Kg.
PITHED 12.30 P.M.
140 mm Hg
120
100
80
60
VI VII VIII IX X XI
7.45 A.M. 8.55 A.M. 9.55 P.M. 10.55 11.55 12.55
0.175 cc. 0.18 cc 0.175 cc 0.137 cc 0.25 cc 0.2
A C A C B B
ZERO PRESSURE

Fig. 2. Shows the relative pressor activities of the three fractions. The pressor activity of 1.0 cc. of A is equivalent to that of 1.29 cc. of B, 0.78 cc. of C.

TABLE 1

Renal assay of material from a previous extraction. Bladder fistula dog. Preparation: No food, no water for 24 hours before experiment

	CONTROL PERIOD			DOSE	FOLLOWING INJECTION					
Urine in 10 minute periods.	1.2 cc.	1.1 cc.	1.45 cc.		0.9 cc.	2.6 cc.	3.7 cc.	1.9 cc.	1.6 cc.	1.6 cc.
Volume urine per minute..	0.125 cc.			1.5 cc.	0.175 cc.		0.28 cc.		0.16 cc.	
Chlorine per cent.....	0.204%				0.508%		0.586%		0.431%	
Chlorine per minute.....	0.255 mgm.				0.89 mgm.		1.64 mgm.		0.69 mgm.	
Urine in 10 minute periods.	1.3 cc.	1.3 cc.	1.5 cc		2.2 cc.	2.9 cc.	2.0 cc.	2.0 cc.	1.0 cc.	1.2 cc.
Volume urine per minute..	0.137 cc.			1.25 cc.	0.255 cc.		0.20 cc.		0.110 cc.	
Chlorine per cent.....	0.356%				0.812%		0.675%		0.50%	
Chlorine per minute.....	0.488 mgm.				2.07 mgm.		1.35 mgm.		0.55 mgm.	
Urine in 10 minute periods.	1.1 cc.	1.1 cc.	1.3 cc.		1.3 cc.	2.5 cc.	1.7 cc.	1.8 cc.	1.5 cc.	1.5 cc.
Volume urine per minute..	0.117 cc.			1.0 cc.	0.19 cc.		0.175 cc.		0.15 cc.	
Chlorine per cent.....	0.48%				0.858%		0.834%		0.523%	
Chlorine per minute.....	0.56 mgm.				1.63 mgm.		1.46 mgm.		0.78 mgm.	
Urine in 10 minute periods.	1.3 cc.	1.4 cc.	1.6 cc.		1.8 cc.	1.7 cc.	1.7 cc.	1.9 cc.	1.5 cc.	1.3 cc.
Volume urine per minute..	0.143 cc.			1.0 cc.	0.175 cc.		0.18 cc.		0.14 cc.	
Chlorine per cent.....	0.465%				0.657%		0.64%		0.457%	
Chlorine per minute.....	0.665 mgm.				1.15 mgm.		1.15 mgm.		0.64 mgm.	
Urine in 10 minute periods.	1.6 cc.	1.6 cc.	1.3 cc.		2.2 cc.	1.2 cc.	1.5 cc.	1.9 cc.	1.6 cc.	1.6 cc.
Volume urine per minute..	0 15 cc.			0.75 cc.	0.17 cc.		0.17 cc.		0.16 cc.	
Chlorine per cent.....	0.18%				0.518%		0.389%		0.306%	
Chlorine per minute.....	0.27 mgm.				0.88 mgm.		0.66 mgm.		0.49 mgm.	
Urine in 10 minute periods.	1.5 cc.	1.8 cc.	1.3 cc.		2.2 cc.	1.6 cc.	2.2 cc.	1.8 cc.	2.0 cc.	2.0 cc.
Urine volume per minute..	0.15 cc.			0.75 cc.	0.19 cc.		0.20 cc.		0.20 cc.	
Chlorine per cent.....	0.32%				0.605%		0.735%		0.695%	
Chlorine per minute.....	0.48 mgm.				1.15 mgm.		1.47 mgm.		1.39 mgm.	

TABLE 1—*Concluded*

	CONTROL PERIOD			DOSE	FOLLOWING INJECTION					
Urine in 10 minute periods.	1.2 cc.	1.3 cc.	1.3 cc.		1.3 cc.	1.5 cc.	1.5 cc.	1.5 cc.	1.6 cc.	1.7 cc.
Urine volume per minute..	0.127 cc.			0.75 cc.	0.14 cc.		0.15 cc.		0.165 cc.	
Chlorine per cent.....	0.53%				0.714%		0.647%		0.57%	
Chlorine per minute.....	0.673 mgm.				1.0 mgm.		0.97 mgm.		0.94 mgm.	

The evidence presented by Abel and his collaborators in favor of the unity of the autacoids of the lobe cannot, therefore, be accepted.

Evidence in support of Abel's "Unitarian" theory has been advanced by Smith and McCloskey (10). These authors found that the pressor and oxytocic constituents of the lobe dialyzed through collodion sacs at equal rates. The renal constituent was also found in the dialysate but, as they were unable to make an assay of this activity, they were unable to state whether or not the diffusion rate was the same as that of the other constituents. The same authors (11) report the parallel destruction of the three activities by heat. Guggenheim (12) found that sodium hydroxide at room temperature destroys both pressor and oxytocic activities. This has also been confirmed by Abel and his collaborators who found that all three activities of their "tartrates" were destroyed at much the same rate by exposure to alkalis at room temperatures, and by boiling with 0.5 per cent hydrochloric acid for half an hour.

The object of these experiments was to repeat the work of Dudley and to extend it so as to include a study of the partition of the renal constituent between normal butyl alcohol and water.

METHODS. *1. Preparation of extract.* Five grams of dried and powdered posterior lobe[2] was defatted by extraction overnight with acetone in a Soxhlet apparatus. The depressor constituent was removed by extraction overnight in the same manner, with ethyl alcohol. The powder, which now weighed 4.3 grams, was extracted with 1000 cc. of water, brought to the boiling point and filtered. The volume of the filtrate was reduced by evaporation in vacuo at 50°C.

After refiltration the volume was made up to 60 cc. and of this 15 cc. were set aside as a control and labelled A. The remaining 45 cc. were then shaken up with 400 cc. of normal butyl alcohol which had been saturated with distilled water. The two layers were then separated by centrifuging and the process repeated three more times. The total volume of normal butyl alcohol employed was 1600 cc. Both layers were evaporated to

[2] Furnished by the kindness of Parke, Davis Co.

dryness in vacuo at 50°C. and the residues taken up in water and filtered. The whole of the alcoholic layer was labelled B and of the aqueous layer $\frac{5}{9}$ was labelled C. The three fractions A, B and C were then diluted to 150 cc. each and after distribution in small bottles were sterilized by heating on the boiling water bath for five minutes on two successive days.

2. Methods of assay. The relative oxytocic activities of the fractions were determined on the isolated guinea pig uterus by the method of Dale and Laidlaw (13). The volume of the bath was in every case 125 cc. and Ringer's solution having the following formula was employed.

	grams
Sodium chloride	9.0
Calcium chloride	0.24
Potassium chloride	0.42
Sodium bicarbonate	0.50
Dextrose	0.50

Aqua destil—ad 1000 cc.

It was found advantageous to reduce the calcium chloride to 0.024 gram per liter when the uterus displayed disturbing spontaneous contractions.

The relative pressor activities of the fractions were determined by the technique of Hogben, Schlapp and MacDonald. In this method pithed cats are used and the injections are made intravenously at intervals of one hour. Under these conditions, the tolerance to the pressor activity exhibited by anesthetized cats is largely eliminated.

The relative renal activities of the fractions were determined on a female dog weighing 12.0 kilos which was kept on a uniform diet. A permanent fistula was established by bringing the bladder out through the abdominal wall and incising it. The edges of the bladder were then sutured to the edges of the abdominal incision. The urine was collected by placing the animal on an operating table with the fistula over a funnel set in the top of the table. To prevent pocketing of the urine in the bladder a flanged glass cannula was inserted into the fistula before each experiment. Under these conditions the urine can be collected, as formed, with great accuracy. One injection only was made during the course of a single experiment. In order, as far as possible, to establish uniform conditions, the animal was deprived of food and water for the twenty-four hours preceding each experiment. No anesthesia was used. The urine excreted during three periods of ten minutes each was collected and set aside as a control. The injection was then made into a leg vein, and the urine collected for one hour following. The urine excreted during each ten-minute period was noted and the total urine excreted during each twenty minutes was set aside separately. Chloride determinations were

TABLE 2

Renal assay of A, B and C. Bladder fistula dog. Preparation. No food, no water for 24 hours before experiment

	CONTROL PERIOD			DOSE	AFTER INJECTION					
Urine in 10 minute periods.	2.6 cc.	2.9 cc.	2.9 cc.	0.2 cc. of A	2.8 cc.	4.0 cc.	3.4 cc.	3.4 cc.	2.8 cc.	
Volume of urine per minute.	0.28 cc.				0.34 cc.		0.34 cc.		0.28 cc.	
Chlorine per cent	0.294%				0.45%		0.38%		0.257%	
Chlorine excreted per minute	0.82 mgm.				1.53 mgm.		1.30 mgm.		0.72 mgm.	
Urine in 10 minute periods.	2.0 cc.	2.2 cc.	2.0 cc.	0.15 cc. of A	1.4 cc.	2.3 cc.	2.4 cc.	2.2 cc.		
Volume of urine per minute.	0.207 cc.				0.185 cc.		0.23 cc.			
Chlorine per cent	0.226%				0.378%		0.278%			
Chlorine excreted per minute	0.468 mgm.				0.70 mgm.		0.64 mgm.			
Urine in 10 minute periods.	2.4 cc.	2.7 cc.	2.7 cc.	0.2 cc. of B	2.6 cc.	3.8 cc.	3.7 cc.	3.9 cc.	3.2 cc.	3.3 cc.
Volume of urine per minute.	0.26 cc.				0.32 cc.		0.38 cc.		0.325 cc.	
Chlorine per cent	0.245%				0.453%		0.345%		0.227%	
Chlorine excreted per minute	0.64 mgm.				1.45 mgm.		1.31 mgm.		0.74 mgm.	
Urine in 10 minute periods.	2.2 cc.	2.2 cc.	2.4 cc.	0.15 cc. of B	2.0 cc.	2.1 cc.	2.6 cc.	3.1 cc.	2.8 cc.	2.6 cc.
Volume of urine per minute.	0.227 cc.				0.205 cc.		0.285 cc.		0.27 cc.	
Chlorine per cent	0.091%				0.142%		0.113%		0.141%	
Chlorine excreted per minute	0.207 mgm.				0.291 mgm.		0.322 mgm.		0.38 mgm.	
Urine in 10 minute periods.	2.1 cc.	2.2 cc.	2.1 cc.	0.15 cc. of C	2.5 cc.	2.7 cc.	2.8 cc.	2.8 cc.	2.5 cc.	2.2 cc.
Volume of urine per minute.	0.213 cc.				0.26 cc.		0.28 cc.		0.235 cc.	
Chlorine per cent	0.163%				0.308%		0.243%		0.171%	
Chlorine excreted per minute	0.347 mgm.				0.80 mgm.		0.68 mgm.		0.40 mgm.	
Urine in 10 minute periods.	2.5 cc.	2.3 cc.	2.4 cc.	0.10 cc. of C	2.3 cc.	2.6 cc.	2.8 cc.	2.6 cc.	2.4 cc.	2.4 cc.
Volume of urine per minute.	0.24 cc.				0.245 cc.		0.27 cc.		0.24 cc.	
Chlorine per cent	0.272%				0.30%		0.31%		0.26%	
Chlorine excreted per minute	0.653 mgm.				0.735 mgm.		0.837 mgm.		0.624 mgm.	

The renal activity of 1.0 cc. of A is thus approximately equivalent to that of 1.0 cc. of B, 0.75 cc. of C.

made on these four fractions of urine by the Volhard-Arnold method. A true pituitary diuresis is always accompanied by an increase in the percentage and total amount of chlorides eliminated and this fact has been utilized as a check against spontaneous changes in the rate of urine excretion. Chlorides appear as chlorine in the tables.

The kidney of the water-deprived animal was found to be extremely sensitive to the extract. A definite diuresis follows the intravenous injection of a dose of the extract which produces submaximal contractions of the isolated guinea-pig uterus. The renal response, under these conditions is sufficiently accurate to detect differences in dosage of 25 per cent.

In the course of a large number of experiments designed to test the accuracy of this method of assay the highest degree of accuracy was obtained when the amount injected was slightly larger than the minimal dose required to produce a definite diuresis. The method employed was to conduct a series of experiments in which the dose injected was reduced by 25 per cent to 35 per cent until the minimal effective diuretic dose was obtained. This helped to eliminate errors dependent upon abnormal responses. The capabilities of this method are illustrated by table 1.

SUMMARY OF RESULTS OF ASSAYS. The following table gives the doses of A, B and C which are equivalent in their oxytocic, pressor and renal activities, respectively.

	PRESSOR	OXYTOCIC	RENAL
	cc.	*cc.*	*cc.*
A	1.0	1.0	1.0
B	1.29	0.263	1.0
C	0.78	10.7	0.75

The following table gives the percentages recovered in B and C of the three activities of the original material employed. It will be noted that in the case of each activity the total recovered in B and C is greater than that of the original material. This discrepancy is due to the error inherent in biological assays. This error[3] may, of course, be cumulative where there fractions are assayed. The ratios of the three activities in the different

[3] The butyl alcohol extraction in this case was made by Prof. R. L. Stehle and the fractions were handed to the writer for assay as "unknowns." The error in the oxytocic assays was, therefore, not realized until the conclusion of the experiment. In two previous extractions carried out by the writer a similar distribution of the three activities was obtained. In both of these cases, however, a considerable loss occurred.

fractions present, however, differences which are much greater than the error involved in the assays.

	PRESSOR	OXYTOCIC	RENAL
	per cent	*per cent*	*per cent*
B	26.0	126.7	33.3
C	77.0	5.6	80.0
Total	103.0	132.3	113.3

Discussion. It has been pointed out by Dudley (14) that absolute proof of the theory that the activities of the lobe are due to one principle must wait upon the isolation of that principle. On the other hand, the extraction of one activity from its solution, leaving the other activities behind, is only possible where the activities are due to separate chemical individuals. The position of Abel and his collaborators, who hold the "unitarian" theory of the autacoids of the lobe, rests on the supposed presence in their highly purified "tartrates" of the activities of the lobe in their original proportions. This evidence, which in any case could only be regarded as presumptive, has been shown to be open to serious criticism.

The work of Dudley and Schlapp and that presented in this paper can only be interpreted by assuming that the autacoid, responsible for the oxytocic activity of the lobe, is chemically separate from the substance or substances responsible for its pressor and renal activities. The renal activity is shown in the tables to follow closely the distribution of the pressor activity during normal butyl alcohol extraction and the data obtained do not warrant a conclusion as to whether the pressor and renal activities are due to one or more than one substance.

The demonstration of the multiplicity of these autacoids must, obviously, have considerable bearing on any attempt to isolate the active constituents of the lobe. The possibility is opened up of obtaining a purely oxytocic drug from the lobe. Such a drug, free from pressor and renal side-reactions, would undoubtedly be valuable in the field of therapeutics. The same may be said of a purely pressor or purely renal drug. A further point of interest lies in the possible deficiency of commercial preparations in these two latter activities.

Acknowledgments. The writer begs to acknowledge his indebtedness to Prof. R. L. Stehle of the Department of Pharmacology of McGill University for constant advice and helpful criticism during the progress of these experiments.

This study has been carried out through the assistance of the A. A. Browne Memorial Fund.

SUMMARY

1. The "unitarian" theory of the autacoids of the posterior lobe put forward by Abel and his collaborators is criticized on the basis of the published results of these authors.

2. The pressor and oxytocic activities are shown to have a widely differing distribution during normal butyl alcohol extraction. The results of Dudley and of Schlapp are thus confirmed.

3. The renal activity is shown to follow closely the pressor activity during normal butyl alcohol extraction.

4. The separation by extraction of the oxytocic activity from the renal and pressor activities is explained by assuming that the autacoid responsible for the oxytocic activity is chemically separate from that responsible for the pressor and renal activities.

5. The data obtained do not warrant a conclusion as to whether the pressor and renal activities are due to one or more than one substance.

6. A method of assaying the renal activity of posterior lobe extracts is described. This method, although time-consuming and possessing a rather large margin of error, may, in the absence of a better one, be of some use in standardizing this activity.

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STUDIES ON THE ANTERIOR LOBE OF THE PITUITARY BODY

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Received for publication September 24, 1926

Since Minkowski (1887) associated acromegaly with a functional disturbance of the pituitary body, much work has been done to determine the activity of this organ in health and disease. It would be out of place to give a complete review of the literature on the pituitary here in view of the able work of Cushing (1912), Bailey (1922), Barker (1922) and Geiling (1926) but reference to some of the work is necessary.

Oliver and Schafer (1895) reported that extracts of the whole pituitary body produced a prolonged rise in blood pressure which was due to constriction of the peripheral arterioles and augmentation of heart beat. Howell (1898) concluded that glycerine and saline extracts of the anterior lobe of the pituitary produced little or no change in the blood pressure and heart rate and that the results of Oliver and Schafer (1895) were due to substances obtained from the posterior lobes of the gland.

Since Howell's work (1898), special attention has been given to the individual lobes of the gland. Much experimental work has been done to determine the effects of extirpation of the whole or part of the gland, feeding the different lobes separately and together and the administration of extracts of the various lobes individually and collectively.

Crowe, Cushing and Homans (1910) reported that complete surgical removal of the whole gland or of the anterior lobe alone resulted in a characteristic train of symptoms termed "cachexia hypophyseopriva," resulting in the death of the animal. They concluded that the anterior lobe or at least a portion of it was essential for the life of the animal. They further observed that removal of the posterior lobe did not result in death but other distinctive symptoms were noted. Camus and Roussy (1923), on the other hand, concluded that the pituitary body is not essential for life of the animals. They further stated that polyuria, permanent diabetes insipidus, rapid genital atrophy and the so-called "cachexia hypophyseopriva" described by others as due to the removal of all or part of the pituitary gland, were due to injury of the hypothalamus during the operations. They were able to produce these symptoms by injuring the hypothalamus without traumatizing the pituitary gland in any way.

Although the work of Camus and Roussy (1923) was well controlled and very thorough, it hardly seems plausible to conclude that the pituitary gland is not of primary importance for the well-being of the animal. The profuse blood supply and histological structure of the anterior lobe, in all vertebrates, would in themselves indicate that this portion of the gland is of some importance.

Goetsch, Cushing and Jacobson (1911) have shown that removal of the pars posterior and pars intermedia of the pituitary gland resulted in a markedly increased carbohydrate tolerance in dogs. They were able to reduce this increased carbohydrate tolerance by administration of extracts of the posterior lobe and to a less extent with extracts of the anterior lobe.

Robertson (1916) prepared a substance from the anterior lobe which he called "tethelin." This substance, he showed, would accelerate the growth of white mice. He further concluded that this substance stimulated rapid growth in primary tumors of carcinomatous rats. This latter observation was similar to those made by Robertson and Burnett (1915) using emulsions of the anterior lobe. Tethelin also proved to be beneficial in the healing of indolent ulcers (Barney, 1917–18).

Evans and Long (1921), Flower et al. (1923) and Evans (1924) reported that anterior lobe extracts given intraperitoneally, produced gigantism and retardation of ovulation in white rats. Growth and maturation of the ova was impaired and oestrus became infrequent or absent.

It would appear from previous work that anterior lobe of the pituitary body supplies a growth-promoting factor. The clinical conditions, gigantism and acromegaly, are both associated with hyperactivity on the part of the anterior lobe of the pituitary body. Borchardt (1908) reported observations on 176 cases of acromegaly. Seventy-one cases of this series showed spontaneous glycosuria which appeared to be independent of the diet. Goetsch, Cushing and Jacobson (1911) confirm this report in that in the early stages of acromegaly spontaneous glycosuria may occur. They suggest that during the hyperplasia of the anterior lobe the pars intermedia and pars posterior may be stimulated to greater secretory activity which in turn produces the glycosuria. They further observed that in the later stages of acromegaly, a marked increase in the carbohydrate tolerance may be present. This increased carbohydrate tolerance was reduced by administration of posterior lobe extracts. These results were similar to those obtained by the same authors (1911) when they showed that the increased carbohydrate tolerance which resulted from the removal of the posterior lobe of the pituitary, could be reduced by administration of extracts of the posterior and anterior lobes separately. These authors did not study the effects of the anterior lobe extracts on the carbohydrate metabolism in normal dogs. In view of Borchardt's report (1908) on the frequent occurrence of glycosuria in cases of

acromegaly and that of Goetsch, Cushing and Jacobson (1911), who showed that anterior lobe extracts reduce the increased carbohydrate tolerance in dogs following the removal of the posterior lobe, the present work was undertaken to observe the changes produced in blood sugar, urine sugar and urine volume in healthy adult dogs, by intramuscular or subcutaneous injections of extracts of the anterior lobe of the pituitary body.

METHODS. Fresh pituitary glands of the ox were used throughout this work. Extracts were made from the anterior lobes and special precautions were taken to insure that only anterior lobe was used. The glands were frozen and a few thin slices taken from the exterior of the anterior lobe, the greater portion of the gland being discarded. There was little likelihood of admixture of the tissue from pars intermedia as pars posterior since Evans (1924) states that, in the pituitary gland of the ox, a separation of the anterior lobe from pars intermedia and pars posterior can be readily effected. Protein-free extracts were made from the thin slices of the anterior lobe thus obtained.

Healthy adult dogs, kept on a constant diet, were used as test animals. These animals were subjected to a control period prior to the administration of the extracts, which lasted for three days. During the control period quantitative determinations of the blood sugar and urine volume were made daily and also the urine was tested for the presence of glucose. Following the control period the extracts were administered to the animals by intramuscular or intravenous injection. During the period of administration of the extracts and the period thereafter, quantitative determinations of the blood sugar, urine sugar and urine volume were made daily until the normal control level was again reached.

Throughout these experiments the blood sugar determinations were made by the Folin-Wu method and the urine sugar determinations by the method of Benedict. The periods of administration lasted from four to ten days, with gradually increasing doses, after which the animals were allowed to recover. In some of the animals as soon as the normal conditions were reached the experiments were repeated.

The action of the extracts on the blood pressure of rabbits and dogs was determined. The blood pressure was recorded by the direct method from the carotid artery and the extracts were injected intravenously by a cannula inserted in the external jugular or femoral vein. The effects of these extracts on excised uterine strips, suspended in oxygenated Locke solution, were also observed. Rabbit and virgin guinea-pig uteri were used.

The livers of the experimental animals were analyzed for glycogen and the results compared with analyses made from livers of normal dogs.

Control experiments were carried out on healthy adult dogs. In these

experiments extracts of fresh beef liver and fresh dog muscle were used. These extracts were made by exactly the same process as the extracts of the pituitary glands. The extracts were administered intramuscularly and records of the blood sugar, urine sugar and urine volume were made as in the experiments described above.

OBSERVATIONS. *a. Changes in the blood sugar, urine sugar and urine volume following the administration of extracts of the anterior lobe of the pituitary body.* The results of these experiments show that, within twenty-

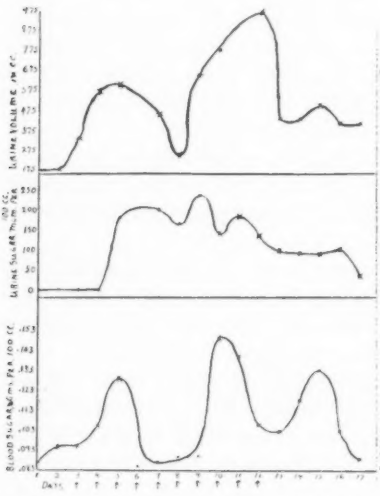

Fig. 1

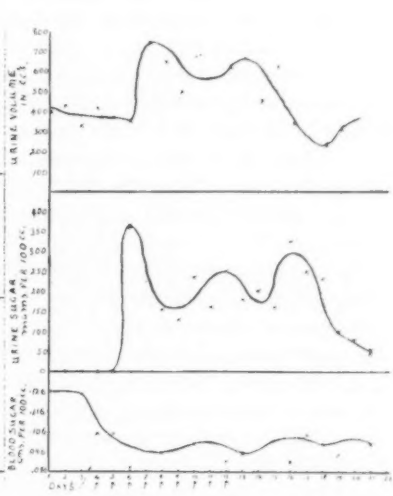

Fig. 2

Fig. 1. Effect of extracts of the anterior lobe of the pituitary body on the blood sugar, urine sugar and urine volume of healthy adult dogs. Graph 1: Changes in blood sugar. Graph 2: Changes in urine sugar. Graph 3: Changes in urine volume.

Fig. 2. Effects of extracts of the anterior lobe of the pituitary body on the blood sugar urine sugar and urine volume of dogs which had been previously treated with the same extracts. Graph 1: Changes in the blood sugar. Graph 2: Changes in the urine sugar. Graph 3: Changes in urine volume.

four hours following the first injection of the extracts, there is a sharp increase in the blood sugar and urine volume accompanied by a marked glycosuria. After several days there is a fall in the blood sugar as well as in the urine volume (fig. 1). The actual amount of sugar in the urine is diminished but the relative amount (mgm. per 100 cc. of urine) remains high. Following this temporary fall in the blood sugar and urine volume a marked hyperglycemia and polyuria develop. The glycosuria which already existed is still pronounced. After the administration of the ex-

tract is discontinued the hyperglycemia, polyuria and glycosuria gradually subside, reaching the control level after several days.

When the experiments are repeated immediately on the same animals the results differ in that hyperglycemia fails to develop as a result of administration of the extract although polyuria and glycosuria develop immediately and remain high throughout the period of administration. Following the period of administration the glycosuria and polyuria gradually subside and reach the normal control period after several days (fig. 2).

b. Effects of extracts of the anterior lobe of the pituitary body on blood pressure. The intravenous injection of the extracts produces a slight rise in the blood pressure of the rabbit and dog. Successive injections of the extracts always produce a pressor effect. The increase in blood pressure due to the second or third injection is just as great as that produced by the first and the records are practically identical.

c. Effects of extracts of the anterior lobe of the pituitary body on excised strips of uterus. The extracts in high concentration fail to stimulate fresh excised strips of rabbit and guinea-pig uterus suspended in Locke solution at 37°C.

d. Control experiments. Effects of extracts of liver and muscle on the blood sugar, urine sugar and urine volume of healthy adults dogs. Intramuscular injections of extracts of liver and muscle failed to produce hyperglycemia, polyuria or glycosuria in any of the animals tested.

Discussion. The function of the anterior lobe of the pituitary body is obscure. As previously stated Minkowski (1887) was the first to associate the condition known as acromegaly with a disturbed function of the pituitary body. At the present time acromegaly and gigantism are associated with pathological disturbances of the anterior lobe of the pituitary. Of Borchardt's 176 cases of acromegaly, 71 showed a spontaneous glycosuria. Extracts made from the whole of the pituitary body, he found, produced a hyperglycemia in rabbits but his results on dogs were uncertain. He concluded that it was suggestive that the glycosurias of acromegaly were due to hyperactivity of the pituitary body.

Goetsch, Cushing and Jacobson (1911), as stated above, confirm the observations of Borchardt in that spontaneous glycosuria is not uncommon in cases of acromegaly. In view of their experimental results on dogs they concluded that the glycosuria of acromegaly was probably due to an active stimulation of the pars intermedia and pars posterior by the hyperplasia of the anterior lobe and later as the disease progresses, with adenoma formation, there is a hypofunction of the posterior lobe and pars intermedia resulting in an increased carbohydrate tolerance.

The present work shows that extracts of the anterior lobe of the pituitary body of the ox produce a hyperglycemia, polyuria and glycosuria in healthy adult dogs within twenty-four hours after the first injection.

The evidence points to the presence of a substance or substances in the anterior lobe of the pituitary which when present in the body, in excess, produce the above symptoms. It therefore seems plausible to infer that the spontaneous glycosuria which occurs in the early stages of acromegaly, may be due to a hyperactivity of the anterior lobe of the pituitary which is, at that time, in a hyperplastic condition.

At this juncture it is of interest to mention the work of Erdheim and Stimme (1909) who observed that, not infrequently, during pregnancy the anterior lobe of the pituitary shows enlargement with marked histological changes. It is of further interest that glycosuria during pregnancy is not uncommon.

The hyperglycemia produced by our extracts is probably due to an increased glycogenolysis. This idea is supported by the observation that the livers of dogs treated with the extracts differed from those of normal dogs in that they failed to show appreciable amounts of glycogen. The observation that hyperglycemia fails to accompany the polyuria and glycosuria in animals which have been previously treated with the extracts is probably due to the increased glycogenolysis which occurred in the liver during the first experiment. The presence of glycosuria and polyuria in the dogs which had been previously treated with the extracts, suggests that the threshold value of the kidney for glucose is lowered as a result of the administration of the extracts. In accordance with this view is the observation made in the first experiments, on normal dogs, that the glycosuria persists when the blood sugar and urine volume are markedly diminished to approximately the normal levels.

The effects of these extracts made from the anterior lobe of the pituitary body, on the blood pressure and on excised strips of rabbit and guinea-pig uterus, indicate that the active substances are not identical with "pituitrin." That the substances are not common to all the tissues of the body is shown by the observation that extracts of liver and muscle, made by the same method, failed to give similar results.

We wish to express our appreciation for the kind suggestions of Prof. F. R. Miller and Prof. A. Bruce Macallum throughout this work.

SUMMARY

1. Extracts of the anterior lobe of the pituitary body of the ox, when injected into healthy adult dogs, cause hyperglycemia, polyuria and glycosuria.

2. Extracts of the anterior lobe of the pituitary body of the ox produces polyuria and glycosuria but no hyperglycemia in dogs which have been previously treated with the extracts.

3. The hyperglycemia is probably due to increased glycogenolysis in the liver.

4. The threshold value of the kidney for glucose, is lowered as a result of the administration of the extracts.

5. The action of the extracts on blood pressure and uterine strips are not similar to the action of "pituitrin."

6. Extracts of liver and muscle made by the same process as the extracts of the anterior lobe of the pituitary body, failed to give similar results when administered to healthy adult dogs.

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THE INFLUENCE OF THE HYDRION CONCENTRATION ON VASCULAR TONICITY

II. With Special Reference to the Dilating Effect of Lactic Acid and Urea

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Received for publication September 29, 1926

It has been observed (Leake, Hall and Koehler, 1923) that buffered phosphate perfusing solutions more alkaline than pH 7.3 or more acid than pH 7.0 tend to constrict the blood vessels of a frog. Moreover, it was found that small amounts of lactate ions (0.15 per cent normal sodium lactate) in such perfusing fluids seem to counteract this constricting action. This so-called specific dilating effect of the lactate-ion was particularly striking in the pH range above 7.4.

Müller (1924) has completely denied this action of the lactate-ion. His experimental observations, however, would not seem to warrant such a sweeping negation. While he found no change in the rate of perfusion when lactate-free fluids were substituted for lactated solutions, or vice-versa, in pH values below 7.6, nevertheless he did note at a pH of 7.6, a dilatation when the change was made from a lactate-free fluid to a solution containing 0.4 per cent lactate-ions, and vice-versa, a constriction.

The studies leading up to this problem have already been reviewed in the two articles noted above. In the work here reported, the effort was made to reach a better understanding of the situation in the light of the pertinent criticisms directed by Müller against the work of Leake, Hall and Koehler.

Critique. Müller rightly criticizes the Americans for failing accurately to adjust the osmotic pressure of their perfusing solutions. He found in the frog that a perfusing fluid (buffered but lacking colloidal material) with an osmotic pressure of $\Delta = 0.55$ or greater tended to dilate the blood vessels, whereas one of $\Delta = 0.50$ or less tended to constrict them. In our experimental work reported here, we adjusted our perfusing solutions in each case to an osmotic pressure of $\Delta = 0.47$, which we considered isotonic with frog's blood. Actual experimentation, however, with perfusing fluids of various osmotic pressures did not convince us that such slight differences as noted by Müller were effective in altering the caliber of the blood ves-

sels. For example, as shown in table 1, only slight alterations in the rate of perfusion, from 2 to 4 seconds per cubic centimeter, resulted from changing an isotonic buffered Ringer's solutions with a pH of 7.21 and Δ = 0.468, to a similar hypotonic fluid with the same pH, but Δ = 0.385, or to a similar hypertonic solution of the same pH, but Δ = 0.592. Inasmuch as we seldom perfused any one fluid longer than 5 minutes, or any one frog longer than 30 minutes, we may not have given time enough for the tendencies observed by Müller to develop.

Careful consideration was given to the method and apparatus proposed by Müller for measuring and recording the rate of perfusion, but it was rejected as inferior to the method previously used by Leake, Hall and

TABLE 1

Effects of altering osmotic pressures of buffered Ringer's perfusing fluids of pH of 7.21 on rate of perfusion in frogs. An increase in the rate of perfusion indicates a constriction of the blood vessels, and vice versa, a dilatation

FROG	OSMOTIC PRESSURE	DURATION OF PERFUSION	RATE OF PERFUSION
	Δ	*minutes*	*seconds per cubic centimeter*
1	0.468	4.5	10
	0.385	5.0	10
	0.592	5.0	12
	0.468	4.0	12
2	0.468	4.0	16
	0.385	4.5	14
	0.592	4.5	16
	0.468	4.0	14
	0.592	4.0	18
	0.385	5.0	18
3	0.468	4.0	16
	0.592	8.0	16
	0.385	8.0	20

Koehler for the following reasons. First, it measures the rate of inflow by drops into a container from which the frog is perfused, thus making possible a considerable error due to conditions beyond the point of measurement. Second, it necessitates an absolutely air-tight compartment in which the drop-recorder is suspended. It is doubtful that this compartment can be maintained air-tight under the conditions prescribed by the type of apparatus. Any slight leak at this point would result in a marked discrepancy between the actual rate of perfusion and the apparent rate as recorded by the dropping apparatus. Third, the ingenious syphon introduced by Müller to regulate automatically the head of pressure presents no advantages over the customary Mariotte pressure bottle system used

in perfusion experiments, but brings in an error due to a probable lag in adjustment.

The rate of perfusion in our experiments was determined in seconds per cubic centimeter, by means of a signal marking pen recording on a revolv-

TABLE 2

Vasodilating effects of lactic acid and urea on the blood vessels of frogs constricted with buffered Ringer's solutions more alkaline than pH 7.3 or more acid than pH 7.0. "R" signifies Ringer's solution; "L" signifies Ringer's solution containing sodium lactate; "U" signifies Ringer's solution containing urea.

Note: The figures shown have been selected (at the suggestion of the editor, and in the effort to save space) from a considerable number of experiments, data from which will be supplied on request.

FROG NUMBER	PERFUSING FLUID AND pH	AVERAGE RATE OF PERFUSION	DURATION OF PERFUSION
		seconds per cubic centimeter	*seconds*
4	R 7.2	26	70
Weight 58 grams	R 7.4	44	83
	R 7.6	72	157
	U 7.6	60	143
	R 7.6	72	167
	L 7.6	42	134
	R 7.6	77	160
	U 7.6	56	178
6	R 7.2	42	90
Weight 35 grams	R 7.4	60	133
	U 7.4	40	104
	L 7.4	36	92
	R 7.4	55	118
	L 7.4	35	85
	U 7.4	39	94
17	R 7.2	14	50
Weight 62 grams	U 6.0	37	122
	R 6.0	44	130
	L 6.0	35	138
	R 7.2	22	105
	R 6.0	38	118
	L 6.0	30	113
	U 6.0	33	125

ing drum the moment of emptying of a double tilting bucket into which the perfused liquid dropped. A thumb-screw adjustment beneath the bucket made it possible to adjust the instrument quite accurately to 1 cc. of the fluid used for the particular temperature at the time. It was also found necessary to correct it in order to account for the practically con-

stant residuum of liquid remaining in the bucket after emptying. When the rate of perfusion was 30 seconds per cubic centimeter, the limits of error of the method were plus or minus 1.4 second per cubic centimeter, as determined by repeated trial using a burette and stop-watch, and with the kymograph revolving at the speed employed in the perfusion experiments.

Experimental. For perfusing fluids we used Ringer's solutions (NaCl 0.65 per cent; KCl 0.014 per cent; $CaCl_2$ 0.012 per cent; $NaHCO_3$ 0.01 per cent) buffered with primary and secondary sodium phosphates, and adjusted by these substances to various pH levels between 6.0 and 7.8. Our lactated perfusates contained 0.15 cc. normal sodium lactate solution per 100 cc. of fluid. Since Hooker (1911) showed that urea added to Ringer's solution and perfused through frogs, caused vasodilatation, we also decided to study the vascular effects of this substance when added to buffered Ringer's solutions, adjusted to definite pH values by means of primary and secondary sodium phosphates. The purpose of this study was to determine whether or not urea, another product of body metabolism, possessed the same vasodilating effects as the lactate ion, when the blood vessels of the frog were constricted by alkaline (pH above 7.3) or acid (pH below 7.0) perfusates. Our urea perfusates contained 0.15 cc. normal urea solution per 100 cc. of fluid. The actual pH levels of our various solutions were determined electrometrically immediately before they were perfused through the frogs.

Leake, Hall and Koehler (1923) perfused each of their frogs with a considerable number of buffered solutions at different pH levels. We determined not to perfuse our frogs for so long a period, but to restrict ourselves to comparing in any one animal the effects of buffered Ringer's solution (R), the lactated fluid (L), and the solution containing urea (U) at the same pH level. Then other frogs were used for comparing the corresponding perfusates at other pH levels. We started perfusion in each case with buffered Ringer's solution at a pH between 7.0 and 7.3, between which points no change in the vascular caliber is ordinarily noted. The brains and spinal cords of the frogs were pithed before perfusion was begun, and the wounds were plugged with cotton.

As may be seen from the tables, and also graphically in figure 1, buffered Ringer's solutions more alkaline than pH of 7.3 or more acid than pH of 7.0 caused a constriction of the blood vessels of the frogs through which they were perfused. The constriction was more pronounced on the alkaline side than on the acid. The addition of small amounts of sodium lactate, or of urea, inhibited this constricting effect, the response again being greater on the alkaline side than on the acid. In comparison with equivalent amounts of urea, sodium lactate was found to be about 10 per cent more active on the average in inhibiting this constricting effect. As

may be noted there was considerable individual variation in the degree of responsiveness to the different perfusing fluids.

Discussion. These findings confirm the results of Leake, Hall and Koehler (1923). They also confirm the results of Hooker (1911) in regard to the dilating action of urea on blood vessels. We find it difficult to understand why Müller (1924) in the face of his own results, should deny the vasodilating action of lactate ions, unless he objects to the use of the word "specific" in connection with it. This vasodilating effect of lactate ions and of urea is "specific" in the sense that it is an active definite effect, and most apparent when the vascular system is constricted by perfusates more alkaline than pH of 7.3 or more acid than pH of 7.0.

We desire to call attention again to the possible physiological significance

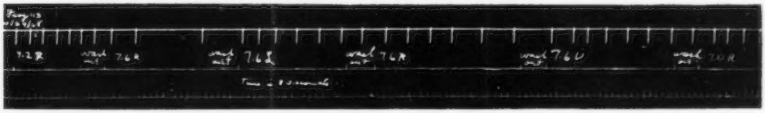

Fig. 1. Graphic record of the perfusion of a frog showing the vasodilating effect of lactic and urea on blood vessels sharply constricted with buffered Ringer's solution at a pH of 7.6. The time is in 8 seconds, and the interval required to change from one perfusing fluid to another is indicated by "wash out." The data from this tracing are as follows:

PERFUSION FLUID AND pH	AVERAGE RATE OF PERFUSION	DURATION PERFUSION
	seconds per cubic centimeter	*seconds*
Ringer's pH 7.2	18	88
Ringer's pH 7.6	59	126
Lactate pH 7.6	25	144
Ringer's pH 7.6	33	186
Urea pH 7.6	25	162

of the dilating effect of small amounts of lactic acid in the fluid passing through a blood vessel. It should be stated here that Dr. F. G. Hall (unpublished data) has found in preliminary experiments that this general discussion, so far as alkaline perfusates are concerned, is applicable to mammals. In addition to the ideas considered by Leake, Hall and Koehler (1923), two other propositions appear worthy of comment.

In the first place, the circulatory changes resulting from anoxemia may be minimized by the concurrently increased lactic acid content of the blood. Anoxemia has been shown by Loevenhart and his pupils (1925) to cause a diphasic shift in blood reaction, with a marked alkalosis developing first. This alkaline condition of the blood might be expected to initiate sharp

vasoconstriction, since the alkalinity may reach a pH of 7.7 and higher. In such an acute anoxemia, Macleod (1921) has found that a considerable increase in the lactic acid content of the blood takes place. Anrep and Cannan (1923) showed further that the lactic acid content of the blood is roughly proportional to the alkalinity, and that it is dependent on the pH of the blood and not, primarily, on the oxygen or carbon-dioxide tension. May not this abnormally high lactic acid content of the blood be concerned in a compensatory mechanism to offset a possibly harmful vasoconstriction due in part to the high alkalinity of the blood in the first stages of anoxemia?

Vasoconstriction in anoxemia is due to augmented blood alkalinity, stimulation of the vasomotor center, and possible outpouring of adrenin. This vasoconstriction is counteracted by the tendency of the increased lactic acid in the blood (also due to the anoxemia) to dilate the blood vessels. The net change in the circulation would tend to remain within physiological limits, but with the blood vessels subjected to an abnormal balance of strongly opposing stimuli, and thus more responsive to any variation in the intensity of these stimuli. Since there is no evidence that increased acidity constricts blood vessels in mammals, these considerations would not necessarily hold in the acid phase of anoxemia.

Another proposition worth comment is the possible rôle of lactic acid in opening up capillaries in a muscle when the muscle becomes active. Krogh (1922) has given beautiful evidence that capillaries are not merely passively dilated by increased blood pressure, but that they possess an active mechanism for changing caliber. It is possible that enough lactic acid escapes oxidation in the initial muscle contraction to come in contact with the capillary tissue, assist in relaxing and opening it up, and thus to permit adequate blood supply to the active muscle.

Increasing data (Heymann, 1921; Hammett, 1922; Evans and Underhill, 1923) is accumulating to show that all smooth muscle is responsive to changes in the pH of the fluid in contact with it. The presence of lactic acid or of urea in such a fluid will apparently alter the character of the response.

SUMMARY

Perfusion experiments in frogs indicate that small amounts of urea as well as lactic acid will cause vascular dilatation when the blood vessels are constricted by buffered Ringer's solutions with a pH above 7.3 or below 7.0. The effect is more striking in the alkaline range.

There is undoubtedly a chemical mechanism of checks and balances for the control of smooth muscle function as well as a corresponding nervous mechanism.

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THE INDUCTION OF PRECOCIOUS SEXUAL MATURITY BY PITUITARY HOMEOTRANSPLANTS

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Received for publication October 7, 1926

The few attempts heretofore made to influence sexual maturity in the normal animal by pituitary transplantation have been uniformly unsuccessful (Schafer, 1911; Clairmont and Ehrlich, 1909; Exner, 1910.)[1] The procedures followed by these investigators were apparently based upon the hope that their transplants would survive for some time, and consequently but a single implant of one or several glands was made. It appeared to me not improbable, judging from my success (Smith, 1926, 1927) in restoring to a normal condition the sex apparatus of animals in which a profound atrophy of this system had been induced by hypophysectomy, that frequent or daily pituitary transplants would be successful in hastening sexual maturity in the normal rat, for the restoration in the hypophysectomized rat was not secured by infrequent implants nor by other methods of pituitary administration (feeding or the injection of fresh bovine pituitary substance). It thus seemed of interest to start a series of experiments in which daily homoplastic pituitary transplants would be made in animals which normally would not attain sexual maturity for many days. Striking results were at once secured: all those phenomena characteristic of sexual maturity (opening of the vagina, the vascular engorgement and the enlargement of the uterus, follicle and corpus formation) being obtained in animals long before they normally would have appeared, and giving results which transcend all normal variability.

That both the genital tract and the ovaries have the capacity to develop precociously appears from the work of others. Thus a sexually mature condition of the vagina and uterus resulted from the injection of follicular

[1] Attempts to hasten sexual maturity by other methods of pituitary administration have likewise been unsuccessful or not convincing, in some cases maturity of the sex apparatus even being apparently delayed. That feeding of pituitary substance does not hasten sexual maturity is clearly shown by the work of Lewis and Miller (1913), Frank (1919), Sisson and Broyles (1921), Evans and Long (1921), Aldrich (1912) and Pearl (1916), whose findings do not support those of Goetsch (1916) and Schafer (1911). The intraperitoneal injection of fresh pituitary substance from the beef does not hasten but apparently retards sexual maturity (Evans and Long, 1921).

fluid in animals only twenty-six days of age (Allen, 1924). The development of the ovaries also can be hastened as shown by the transplantations of Evans and Long (1922). Ovaries from immature rats rapidly matured and induced oestrus when transplanted into adults. It is thus evident that the maturity of the genital system depends not upon an age factor alone but also upon the presence or absence of certain constituents in the body fluids.

METHOD. In this study only young female rats (pied) have been used as recipients for the transplants. The pituitary tissue for transplantation was secured indifferently from full-grown male and female rats. The posterior lobe was usually included in the transplant although the same effects were obtained if the pars anterior alone, was used. The method of transplantation is simple and rapid. After decapitating the donor, its cranium is opened and the pituitary exposed, care being taken to preserve its sterile condition. The gland is then cut into several fragments, drawn into a small glass cannula and injected into the leg muscle of the anesthetized recipient. The entire process aside from anesthetizing the recipient takes but a few minutes. One transplant daily was made in all cases.

The vagina was allowed to open spontaneously. In certain cases autopsy was done immediately after the complete establishment of the vaginal canal. In other cases it was postponed for several days.

In addition to the giving of transplants to normal immature females, in several cases similar treatment was accorded to immature ovariectomized females. In all approximately thirty animals have been given transplants, littermate sisters being used in all cases as controls.

RESULTS.—Reference to table 1 shows the rapidity with which sexual maturity is induced by the pituitary transplants in animals which otherwise would have remained sexually immature for a considerable period.[2] Four to nine transplants, depending upon the age of the recipient, invariably caused the complete establishment of the vaginal canal, a uterine hyperemia together with an oestral accumulation of fluid, and the formation of large follicles or of corpora lutea. Cornified epithelium appeared in the vagina immediately or within a few hours, as shown by smears. This condition was produced as early as the weaning date (on the 22nd day of

[2] "Sexual maturity" is used here in the same sense as used by Allen and Doisy (1924) and is synonymous with puberty. It was used by Long and Evans (1922) to designate the time when mating first took place. Such a use here appears inadvisable, for the small size of these animals makes copulation as a test of sexual maturity uncertain.

There is great variation in the age at which sexual maturity normally takes place. Long and Evans (1922) found in a group of 200 rats that the age at first ovulation varied from 45 to 147 days, averaging 77 days; the age at which the vagina opened varied from 34 to 109 days, averaging 72 days. In another group of 600 rats 2 ovulated at 35 days.

TABLE 1

The table gives the age at which the daily pituitary transplants were commenced and the number given, also the age at which the vagina opened and at which autopsy was performed with weights of the genital organs

DESIGNATION OF RAT	EXPERIMENT STARTED				EXPERIMENT TERMINATED—AUTOPSY								
	Received transplants		Controls, no transplants		Age	Weight	Vagina opened age	Number of days transplants given	Weight of		Type of uterus	Ovaries	
	Age	Weight	Age	Weight					Both ovaries	Uterus and tubes		Corpora	Follicles
	days	*grams*											
GH 1467	23	49			29	71	29	6	0.0190	0.109	Oestrus	Absent	Small and medium
W 1468	23	49			33	83	29	10	0.0740	0.225	Oestrus	Present	Small and medium
BH 1471	23	48			33	75	29	10	0.0750	0.189	Oestrus	Present	Small and medium
W 1469			23	49	29	73	Not open		0.0150	0.031	Slender	Absent	Small
W 1470			23	49	52	142	Not open		0.0195	0.056	Slender	Absent	Small and medium
GH 1472			23	49	52	141	52		0.0215	0.323	Oestrus	Absent	Small and medium
GH 1473			23	47	52	123	51		0.0255	0.103	Slender	Present	Small and medium
GH 1485	20	37			43	119	26	8	0.0190	0.134	Slender	Few	Small. Much interstitial tissue
BH 1486	20	39			43	132	26	8	0.1140	0.130	Slender	Many	Small. Much interstitial tissue
GH 1490	20	42			28	54	24	7	0.1070	0.134	Medium	Many	Small
BH 1487			20	41	43	106	Not open		0.0130	0.050	Slender	Absent	Small and medium. Much interstitial tissue
BH 1488			20	41	43	102	43		0.0198	0.183	Oestrus	Absent	Large
BH 1489			20	45	43	107	40		0.0210	0.226	Oestrus	Present	Large
BH 1491			20	37	43	105	38		0.0200	0.097	Slender	Present	Small
W 1496	14	25			22	41	22	8	0.0260	0.154	Oestrus	Absent	Medium
W 1495	14	27			24	47	24	10	0.0550	0.133	Oestrus	In left only	Medium. Photo
W 1497	14	26			24	44	Not open	10	0.0260	0.142	Oestrus	Absent	Large
W 1494			14	26	22	41	Not open		0.0098	0.029	Slender	Absent	Small
W 1492			14	27	24	47	Not open		0.0110	0.025	Slender	Absent	Small
W 1493			14	27	24	47	Not open		0.0142	0.024	Slender	Absent	Small. Photo
GH 1513	21	47			29	60	27	5	0.0500	0.117	Oestrus	Present	Large
W 1514*	21	47			29	62	Not open	5		0.028	Slender		
W 1516			21	45	29	59	Not open		0.0170	0.033	Slender	Absent	Small

W	1640†	26	32			32	41	31	5	0.0198	0.267	Oestrus	Absent	Large
W	1642	26	35			32	44	31	5	0.0286	0.305	Oestrus	Absent	Large
W	1637			26	35	32	48	Not open		0.0135	0.043	Slender	Absent	Small
W	1639			26	36	32	51	Not open		0.0150	0.052	Slender	Absent	Small
W	1638*	26	34			32	42	Not open	5		0.0238	Slender		
W	1641*	26	32			32	40	Not open	5		0.0260	Slender		

* Ovariectomized two days before transplants were commenced.

† This animal received anterior lobe transplants only.

life), transplants having been commenced in this case at the age of 14 days (table 1). The gross appearance of the sex apparatus of an animal which received the transplants as contrasted with an untreated control is most striking (figs. 1, 2). It impresses one with the potency of the pituitary tissue, the implantation of which produced these profound changes.

An autopsy performed immediately after the opening of the vagina revealed the presence of large follicles but no corpora. However, the postponement of autopsy for a few hours invariably showed the presence of corpora. The changes occurring in the ovaries of these precociously mature females thus correspond to those normally occurring at sexual maturity.

The history of the few animals which were allowed to survive for a period following the attainment of this precocious maturity and which received no more transplants, shows that the characteristic oestral, vaginal smear soon changed to the dioestral type. This type of smear persisted in the two animals allowed to survive for 17 days after the opening of the vagina, and in a number not autopsied for 10 days. At autopsy their uteri were in the dioestral condition. This postponement of the second oestral cycle which is longer than that usually displayed by the animal which is allowed to mature normally, together with the failure of the body and organ weights to show any marked increase over that of their normal controls indicates that general body development has not been greatly hastened by the transplants.

In no case has mating of these animals at the appearance of oestrus been attempted. Such a test appeared futile because of the difference in size obtaining between them and the sexually mature male. Furthermore such a test would not appear to be of much significance since mating does not usually occur at the first oestrus in the normal animal (Long and Evans, 1922).

It was of some interest to determine whether the time required to induce

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Figures 1 to 8 are from rats W 1495 and W 1493 (table 1).

W 1495—Received daily pituitary transplants from the fourteenth to the twenty-third day of its life (10 transplants). Mature sexually. Autopsied at the age of 24 days.

W 1493—Littermate sister control. Immature sexually. No transplants. Autopsied at the age of 24 days.

Fig. 1. W 1495. Uterus showing enlargement and vascular engorgement. × $1\frac{3}{5}$.

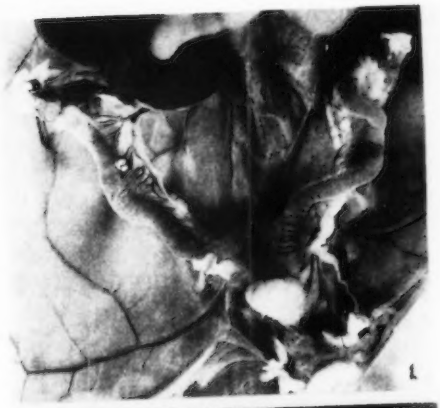
Fig. 2. W 1493. Uterus showing immature condition. × $1\frac{3}{5}$.

Fig. 3. W 1495. Epithelium of vagina. Upper third. × 240.

Fig. 4. W 1493. Epithelium of vagina. Upper third. × 240.

Fig. 5. W 1495. Middle third of left horn of uterus. The uterine fluid escaped during the time taken to photograph the intact sex apparatus and the organ contracted. × 61.

Fig. 6. W 1493. Middle third of left horn of uterus. × 61.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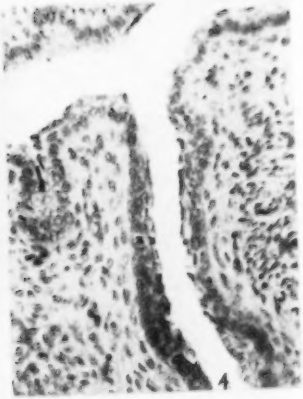
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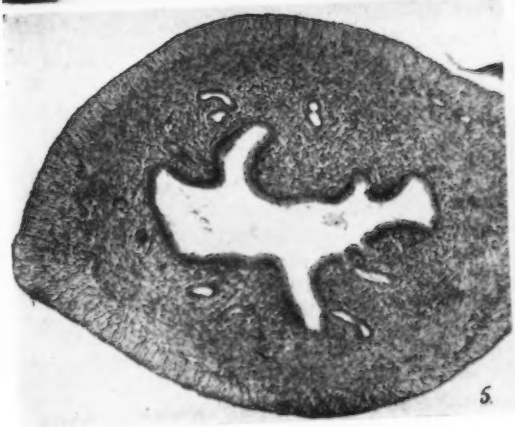
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premature sexual maturity depended solely upon the number of transplants given or whether the age of the recipient also played a part. In one series daily transplants were begun when the recipients were 14 days of age. The vagina opened in one animal on the 9th day following, in two on the 11th day. In other series in which the transplants were not begun until the recipients were 20 to 26 days of age sexual maturity was induced in from 5 to 7 days (table 1). Thus within broad limits the age of the recipient appears to play a part in the time taken by the transplants to induce sexual maturity.

Thus far I have considered only the induction of premature sexual maturity in the normal rat by the daily pituitary transplants. The opposite condition, a postponement of the complete establishment of the vaginal canal, results from pituitary ablation. Although my data on this point are not extensive due to the fact that most of my hypophysectomies have

TABLE 2

Table showing the effect of hypophysectomy before sexual maturity upon the opening of the vaginal orifice in the rat

DESIGNATION AND TYPE OF RAT	EXPERIMENT BEGUN*		VAGINA OPENED	
	Age	Weight	Age	Weight
W 1453 Normal control	42	99	44	107
W 1455 Hypophysectomized	42	107	114	90
W 1456 Hypophysectomized	42	99	46	89
B 1457 Hypophysectomized	42	113	Not open on 156th day	93

* The hypophysectomized animals were operated upon at the age and weight given in this column.

been done at about the time of sexual maturity or later, yet the few cases available indicated rather conclusively that pituitary ablation postpones the opening of the vagina (table 2).[3] In several animals whose vaginal orifices failed to open following pituitary ablation I have made daily pituitary transplants. In no case has this treatment failed to open the vagina and to induce the uterine and vaginal changes characteristic of oestrus.

A microscopic study of the genital system of the animals which were

[3] The cyclic changes of the genital apparatus which occur with great regularity in the normal female are never seen in the hypophysectomized rat, consequently they could not be used to determine sexual maturity. As a matter of fact the hypophysectomized rat never matures sexually. It was interesting to find that the ablation of the pituitary will inhibit the establishment of the vaginal orifice, an effect which does not follow ovariectomy (Long and Evans, 1922).

given the daily pituitary transplants shows that this treatment induces all the structural changes characteristic of sexual maturity, while the untreated littermate sisters exhibit an immature condition. In the treated animal the vaginal epithelium is many-layered, the surface cells are cornified and frequently dehiscent (fig. 3) in contrast to the two or three cell layers forming the epithelium in the control (fig. 4). The uterus of the treated animal is lined by a high columnar epithelium beneath which there is a well differentiated submucosa, under which are well developed muscular layers (fig. 5). In the control the uterine epithelium is low, the submucosa undifferentiated and the muscular coats neither well developed nor

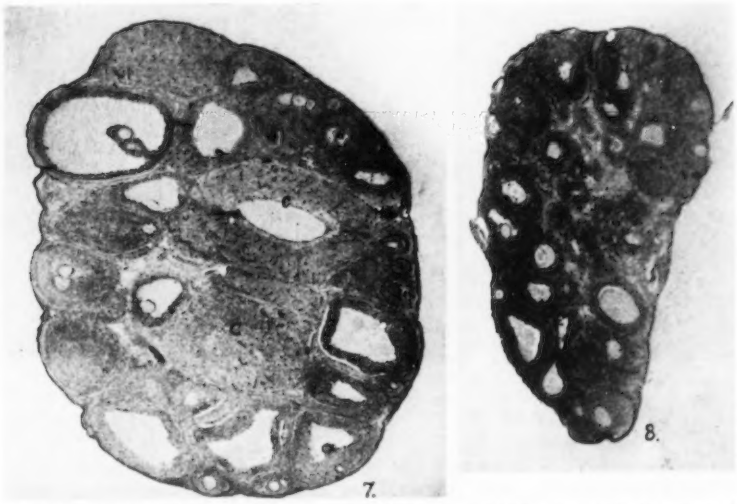

Fig. 7. W 1495. Left ovary. The section is through the greatest diameter of the gland. Corpora are present. c. × 30.

Fig. 8. W 1493. Left ovary. Section is through the greatest diameter of the gland. × 30.

well differentiated (fig. 6). The ovaries of the treated animals show a mature condition. Follicles of all ages, most of which display no degenerative changes, are present and in all those cases in which a sufficient interval of time separated the opening of the vagina and autopsy, corpora are present (fig. 7). These usually have a central cavity as is generally the case in newly formed corpora lutea. In contrast to this condition is the picture displayed by the ovaries of the untreated littermate sisters. The follicles present are small and frequently atretic and there are no corpora (fig. 8). It is thus seen that structurally all the criteria of sexual maturity are fulfilled by these treated animals.

Kymographic records of the spontaneous contractions of the isolated uterus in Ringer's solution show that the amplitude of contraction of this organ, in the treated rat, approaches or equals those from animals which have matured at the usual time. They greatly transcend the amplitude

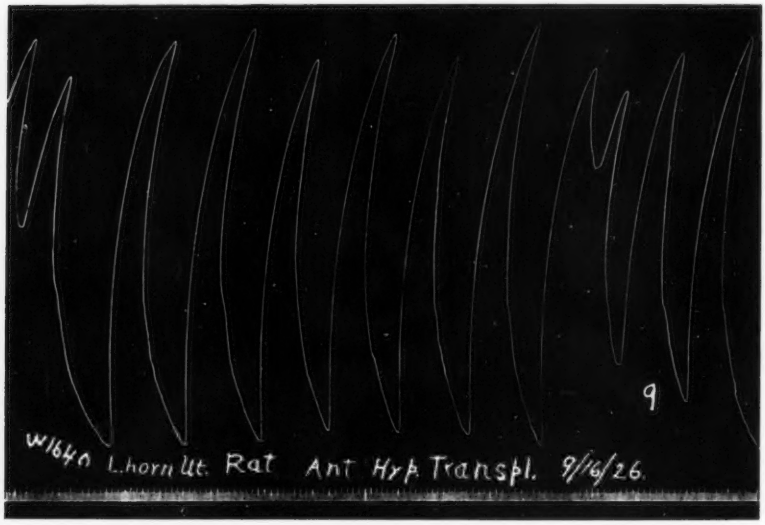

Fig. 9. W 1640. Matured sexually at the age of 31 days after receiving anterior pituitary transplants for 5 days. Kymographic record of the uterine contractions. See table 1.

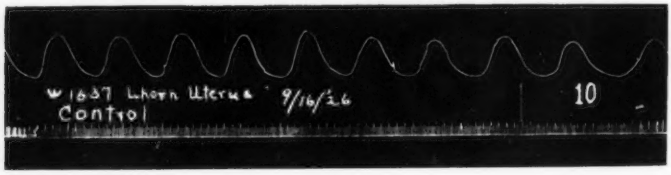

Fig. 10. W 1637. Untreated littermate control. The length of the recording lever and its load were the same in making these two records.

of contraction displayed by the uteri of the controls (figs. 9, 10). Thus, as far as can be ascertained, by this method there is a profound physiological difference between the uteri of the controls and treated rats.

It seemed of interest to determine whether pituitary transplants would stimulate the development of the uterus and vagina of the immature rat,

in the absence of the ovaries. Three animals were ovariectomized near the weaning date, and after waiting two days for recovery from the operation, they together with unoperated litter mates were given daily pituitary transplants, untreated littermate sisters serving as controls. Not only were none of the characteristic phenomena of oestrus produced by these transplants in the absence of the ovaries but the uterine atrophy which follows ovariectomy was not prevented (table 1). This finding is in harmony with and perhaps could be anticipated from the work of Allen and Doisy (1924) in which it was found that precocious maturity resulted from the injection of follicular fluid. The fact that the vagina opens at the normal time in the absence of the ovaries (Long and Evans, 1922) gave me some hope, however, that these transplants might be effective in causing a premature opening in the absence of these glands. Success seemed not improbable, also, from the fact that the uterine atrophy consequent to hypophysectomy is greater than after ovariectomy, a difference which shows that the ovarian atrophy which is induced by hypophysectomy is not solely responsible for the accompanying uterine atrophy.

Discussion. The success of the writer in invariably inducing premature sexual maturity by daily pituitary transplants stands in sharp contrast to the failure of other methods of pituitary administration to hasten the development of the sex apparatus of the immature animal. The failure of other investigators to secure this hastening by pituitary transplants is readily understandable for but one transplant—of one or several glands—was made in each animal. This soon degenerated as shown by the histological study of Clairmont and Ehrlich (1909). In my work this degeneration has not made the treatment ineffective because the transplant was daily renewed. The failure of pituitary feeding to hasten sexual maturity is not surprising for with this method of administration the digestive process as well as possible autolysis from the use of not absolutely fresh tissue may injure the hormone. The failure of the intraperitoneal injection of the bovine pituitary suspension of Evans and Long (1921) to hasten sexual maturity is more surprising. One reason for this failure might be sought in a functional difference between the pituitary of the rat and of the beef, although any such marked functional difference between the pituitary of these two mammals is improbable. The cause of the failure would seem much more likely to be due to changes in the gland before it was used, for although their pituitary tissue was fresh as compared to the dried gland, it nevertheless was several hours old. Many changes in the hormone could take place in this period, changes to which the method of preparation may also contribute.

From the failure of the uterus and the vagina to respond to the transplants in the absence of the ovaries; from the precocious sexual maturity induced by the injection of follicular fluid (Allen and Doisy, 1924); and

from the follicular growth which results from the pituitary transplants, it seems clear that the hastening in the maturity of the uterus and the vagina by pituitary transplants, is due to the presence of the follicular hormone the formation of which has been stimulated by these transplants.

Most recent investigators maintain that the ablation of the pituitary has no effect upon sexual development or function. Their results—secured mostly in the dog—are not in conformity with mine secured in the rat, for I have found that after pituitary ablation the sex apparatus not only fails to develop but undergoes an actual atrophy. The invariable and speedy maturity of the sex apparatus which is induced in the normal immature rat by homoplastic pituitary transplants supports the idea of an hypophyseal-gonadal interrelationship and speaks against the views of those who maintain that the maturity and function of the sex apparatus are independent of the pituitary.

SUMMARY

1. Daily homoplastic transplants of the pituitary from the adult, given intramuscularly, induce sexual maturity in the female rat as early as the weaning date (22 days of age), thus transcending all normal variability. Animals which are but fourteen days of age when treatment is begun mature sexually in from eight to ten days; when the treatment is begun at the weaning date they mature in five or six days.

The opposite response of the sex apparatus results from pituitary ablation before sexual maturity, the complete establishment of the vaginal canal being delayed and the genital system remaining undeveloped.

2. The anterior pituitary component of the transplant only, is essential for the induction of premature sexual maturity, the posterior lobe of the pituitary neither hindering nor aiding in the response.

3. No stimulus to the development of the uterus or vagina of the immature ovariectomized rat is given by these transplants.

4. The invariable and rapid maturity which is induced by daily pituitary transplants speaks against the view of those who maintain that hypophysectomy does not interfere with sexual development or function, and indicates an important hypophyseal-gonadal interrelationship.

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CHANGES OF ELECTRICAL CONDUCTIVITY IN THE BEATING TORTOISE VENTRICLE

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Received for publication October 11, 1926

The present day theory of stimulation is based on the conception of Bernstein (1912), and involves the idea of the presence of semi-permeable membranes, which are, when the tissue is at rest, in a state of polarization. Upon stimulation, it is supposed that this resting polarization is destroyed as a result of the breakdown of the membrane at the point of stimulation. Considerable evidence has been accumulated in recent years to indicate that activity in a tissue may be associated with increase of membrane permeability. This conclusion has been arrived at by investigators employing differing methods of attack. For example, Mitchell, Wilson and Stanton (1921) have shown that frog's muscle perfused with Ringer's solution in which potassium has been replaced by rubidium or caesium takes up the latter substances when the muscle is stimulated during the perfusion, but not when the muscle is at rest. Crozier (1922) has observed that the penetration of acid into the mantle tissue of *Chromodoris zebra* is accelerated after local stimulation, and Lillie (1916) has noted an increased permeability during the cleavage of echinoderm eggs.

It has, moreover, been observed that substances which increase permeability are also stimulators of activity. Thus, Loeb (1913) has shown that many such compounds of widely differing chemical composition—soaps, alkaloids, glucosides, etc.—activate artificial parthenogenesis in arbacia eggs, and Lillie has seen the same phenomenon upon placing starfish eggs in pure NaCl, a salt which Osterhout (1922) has shown to increase membrane permeability.

Other observations have been made using electrical conductivity as an index of permeability. McClendon (1912) has published a short note indicating an increase in the conductivity of frog skeletal muscle during contraction. He does not describe his method in detail, but one gathers that he determined the resistance of the muscle at rest, and again at some point during tetanization. The influence of the stimulating current, apart from the heating effect, was disregarded, as was a change in the form of the muscle, although the contraction was apparently not isometric.

Recently Bronk and Gesell (1926) have measured the electrical conductivity of the submaxillary gland, and have demonstrated that when visible secretion was elicited as a result of stimulation the conductivity invariably decreased. They have explained this apparent contradiction of earlier results by assuming that there is an increase of permeability of the membrane on the lumen side of the cells and a decrease on the basement side. If the latter were greater than the former, the net result would be a decrease in conductivity.

At the Stockholm Congress, Peserico (1926) also reported experiments on the submaxillary gland, in which he found that after a short latent period following stimulation of the chorda tympani, there was a period of increased conductivity, followed, as in the observation of Bronk and Gesell, by a decrease coinciding with the secretion of saliva.

It seemed to us that further study of the relation of activity in organs to changes in their electrical conductivity was desirable. The experiments which we are reporting in the present paper have to do with this relation in the case of the beating tortoise heart. The heart lends itself peculiarly well to such a study for a number of reasons. It is easy, for one thing, to introduce a barrier of tissue between the conductivity electrodes in such manner that all of the current must of necessity flow only through the tissue itself. Again, the automaticity of the cardiac contraction removes the complicating factor of external stimulation. It is moreover not difficult to produce and record isometric contractions, involving no change in the length and thickness of the muscular wall. By adapting to our needs the ordinary methods of observing and recording changes of conductivity, and correlating these with simultaneous variations in intraventricular pressure, we have obtained results indicating, in brief, that ventricular systole is associated with an increase in the electrical conductivity, which returns to normal during diastole and diastasis.

A question should arise at this point as to the relation between changes in electrical conductivity and changes in membrane permeability. In other words, is one justified in speaking of an increase in membrane permeability when one is actually measuring the electrical resistance of the tissue as a whole? It is true, for one thing, that in doing so we disregard any condenser action in the tissue, which may influence any quantitative deductions that may be drawn. Were this paper to deal with quantitative estimations, this objection might be sound, but we have been compelled, for reasons into which we shall enter later, to restrict our conclusions largely to qualitative ones, and to make our quantitations only approximate.

Another and more disturbing objection relates to the possible effect of molecular reorientation within the cell as a factor in changing the electrical resistance. In a recent paper, Garner (1925) has advanced a theory of muscular contraction which involves not only a change in membranes, but

also in the reorientation of molecules in the hypothetical liquid crystal units which are bounded by these membranes. That such a reorientation may occur accompanying contraction is of course conceivable, and while it is commonly held that changes in electrical conductivity are a function of changes in membrane permeability (under the conditions of such experiments as ours) the possibility is to be conceded that the phenomena to which we have alluded must be taken into account.

APPARATUS. In order to study the comparatively rapid changes in electrical conductivity taking place in the heart it was necessary to deviate from the usual method of determining and recording changes in resistance. The basic portion of the apparatus was the usual Wheatstone Bridge, the modifications being made to the end that optical records of sound

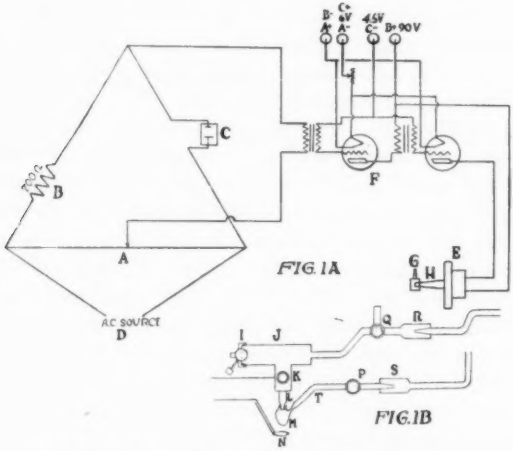

Fig. 1. Diagram of conductivity apparatus

changes and intraventricular pressure might be recorded simultaneously. Figure 1 presents the apparatus that we used. *A* is a 1000 mm. slide wire resistance connected at the ends to a variable resistance *B*, which was set at 700 ohms in the majority of our experiments, and to the conductivity cell *C* in which the heart was placed; *C* will be described in detail later. *B* and *C*, in turn, were connected and led to the primary of the first transformer of the low frequency amplifier *F*. The other pole of the primary was connected to the sliding contact of resistance *A*. The ends of the slide wire *A* were also connected to the alternating current source.

We were obliged to use an alternating current of lower frequency than the 1000 cycles commonly employed in conductivity measurements because of the low vibration frequency of our sound recording apparatus. We dis-

carded, after a few experiments, an electric tuning fork of 100 vibrations on account of the difficulty of amplifying such a low frequency and in view of the irregularities of the current evolved. A vacuum tube oscillator, as described by Hunter and Patrick (1925) with the substitution of 0.1 for 1 microfarad condensers (which gave a frequency of 300 cycles) was likewise discarded owing to the impossibility of matching the impedance of the induction coils of the oscillator with that of the bridge. The current source that we finally found to suit our necessities was a small induction coil with 6 volts on the primary and a 2 mf. condenser across the vibrator to prevent sparking. This gave us a frequency of 200 to 300 cycles.

The transformer, the primary of which was connected in place of the ordinary telephone receiver, was the first unit of a two stage low frequency amplifier, *F*. The first transformer had a ratio of 6:1, the second 3.5:1. Both were of the iron core type. The vacuum tubes were type U.V. 201A, with 5 volts heating the filament and 90 volts impressed on the plate. The grid bias was 4.5 volts.

The amplifying unit in turn was connected to a large telephone receiver *E*. To this was joined by a short length of glass and rubber tubing the first portion of the sound recording apparatus, a sound capsule devised by Wiggers and Dean (1917). The vibrating membrane of this capsule was made of a thin film of rubber cement, allowed to dry, and having on its surface a small mirror. By making many membranes it was possible to obtain one that was sufficiently sensitive for our purpose. The frequency of the current was further adjusted to the resonance of the membrane by minor adjustments of the vibrator of the induction coil. While the making of films that were sufficiently taut and durable was decidedly uncertain and laborious we were able to obtain membranes which at a frequency of about 250 cycles would give a change of as much as 12 mm. breadth of light beam for every millimeter the sliding contact moved away from the zero position. While it was possible to make membranes of still greater natural frequency it was our experience that they were too fragile to be serviceable.

The heart was mounted in our apparatus in what served at once as a conductivity cell, as a perfusion cannula, and as a manometer for recording optically the changes in intraventricular pressure (*C* in fig. 1 A, shown in detail in fig. 1 B). The main portion of the cell, if such it may be termed, consisted of a T tube of $\frac{7}{8}$ inch glass tubing, in the vertical arm of which was sealed one electrode *K*. This arm terminated in a cannula *L* (with a lumen of about 3 mm.) which was inserted in the auriculo-ventricular orifice. At one end of the horizontal portion of the T-tube the pressure segment capsule was attached. The elastic portion of the capsule was a piece of rubber dam. This was aligned with the sound capsule, which was placed directly above. At the other end of the horizontal arm of the T was attached a 3-way stopcock, *Q*, which was bent at such an angle as to

permit the filling of the apparatus without bubbles. The stopcock led to an inflow valve, *R*, which in turn was connected to a reservoir of 0.65 per cent saline. A second cannula *T* was inserted in the ventricular cavity through one of the large arteries, and connected to a 2-way stopcock, *P*, which in turn led to an outflow valve *S* through which the saline was conducted from the reservoir. The valves were of the Sadd type.

Illumination of the mirrors of the sound and pressure segment capsules was by means of an arc lamp with the double slit attachment of Katz and Baker (1924). Control measurements proved that our arrangement of light and capsules was free from parallax.

All connections were made by bringing glass to glass and holding this in place by heavy rubber tubing, particularly in those joints which occurred between the stopcocks and the cannulae. This was done in order that the contraction, save for the minute movements of the pressure capsule membrane, might be isometric when the cocks were closed.

The second electrode, *N*, was placed directly beneath the heart and as close to it as possible. Both were then immersed in a beaker of saline. The electrodes were approximately 8 cm. apart.

Technique. In order that the heart might have a fresh supply of saline the stopcocks of the perfusion apparatus were left open until the tissue had been well washed. With the stopcocks closed a determination of the resistance of the conductivity cell (heart and saline) was made during diastasis. The sliding contact of the wire was then moved 2 to 5 mm. to the right or left and a photograph taken of the pressure and sound changes. As the sound changed in the telephone receiver, the vibration of the capsule varied in the same manner, i.e., with an increased sound one obtained a band of greater width on the bromide paper. Following a reading on one side of the zero for diastasis, a second was taken on the opposite side at the same distance from the zero.

This procedure was necessary because it was of course impossible, in view of the rapidity of the change, to determine the "zero" point for successive phases of the contraction by moving the sliding contact. It has the drawback that quantitation of the results can only be approximate. This will be referred to later.

The results of this measurement may be interpreted as follows. If the sliding contact is set to the right of the zero of diastasis and we find during contraction a decrease in the width of the sound beam, it is obvious the zero has approached the point at which the sliding contact is set. Conversely an increase in sound would indicate movement of the zero point in the opposite direction. Such a movement to the right of the original zero would, according to the formula for the Wheatstone bridge, represent a decrease in the unknown resistance, in our case the heart. Hence, such a

state, which actually occurs as will be shown later, will mean an increase in conductivity during contraction.

When the isometric changes had been recorded the stopcocks were opened and the zero for isotonic diastasis determined. This redetermination of the resistance was obligatory as it was not necessarily the same as for isometric diastasis. After this, observations were made similar to those in the case of the isometric contraction.

EXPERIMENTAL RESULTS. *1. The isometric contraction.* Figure 2 shows photographs of isometric contractions recorded by the method we have described. Numbers 1 and 2 are records taken on opposite sides of the "zero point" in a single beat; in the former, greater conductivity is repre-

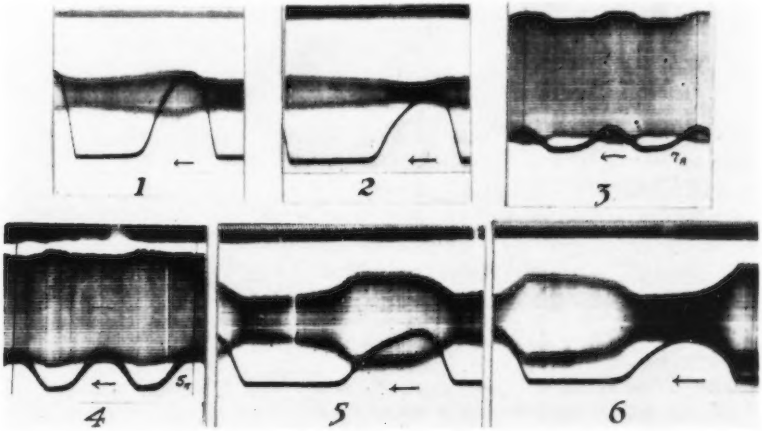

Fig. 2. Photographic records of isometric contractions. Lower line represents intraventricular pressure.

sented by an increase, in the latter by a decrease in the sound. It will be seen that during systole the conductivity becomes greater, reaching a maximum after the development of the greatest intraventricular pressure. During diastole and diastasis the conductivity becomes less. Numbers 3 and 4 are similar records in another heart. In spite of the fact that these show what seem to be much smaller changes than numbers 1 and 2, the magnitude of the resistance variations in the two cases is of the same order, the apparently considerable difference between them being due to differences in the sensitivity of the sound capsules assembled on these particular days. Numbers 3 and 4 are records of a heart in which the increase in conductivity did not reach a maximum until an exceptionally long interval after the peak of intraventricular pressure.

Figure 3 shows the average percentage change in the sound width in relation to concomitant changes in the curve of intraventricular pressure during isometric contraction. The sound changes above the 100 per cent line were observed when the slider of the Wheatstone bridge was at the right of zero; those below the 100 per cent line, when it was at the left of zero. An increase in sound width, in the former, and a decrease, in the latter (as explained under "Technique") indicated an increase in the electrical conductivity. All of the beats used in making this average curve were reduced to a standard time of 6 seconds. The changes in conductivity are represented in this figure as percentages of the sound width at the beginning of the rise in intraventricular pressure, while the latter is given as the height in millimeters from the base line, as projected and enlarged.[1] The results shown are hence qualitative, and are merely to be taken as differences in the intensity of these two phenomena in relation to time. The summarized data which form the basis of the curves in figure 3 are given in table 1.

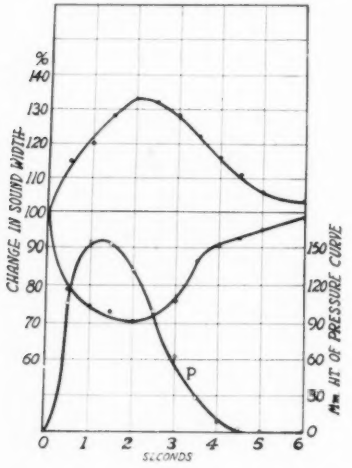

Fig. 3. Conductivity changes in relation to intraventricular pressure changes. Curve *P* represents intraventricular pressure, in the sound curves, the one above the 100 per cent line is taken at the left of "zero." In this curve, an *increase* in sound width indicates an increase in conductivity. In the other sound curve, taken at the right of zero a *decrease* in sound width indicates an increase in conductivity.

It is evident that beginning with the onset of systole there is an increase in the electrical conductivity of the ventricle. In a preliminary report on this matter (1926) we stated that the change in conductivity actually preceded the mechanical evidence of contraction. Were this point fully established, it

[1] Since the method we have employed for the measurements of our photographs has not been hitherto described, a brief mention at this point may not be amiss. The tracing to be analyzed was placed in a photographic printing frame and projected by a Bausch and Lomb "combined Balopticon." This apparatus was so arranged that the image was projected down to the surface of a drawing board holding a large piece of cross section paper. The distances used in our arrangement (35 inches between the centers of the mirrors which were 35 inches above the table top) gave a magnification of about 3 times. It was found convenient to use a 10 by 20 inch first surface mirror as the second reflecting surface in order that the entire beat be visible at the same time. This method facilitated the tracing and measurement of the sound and pressure curves.

would be of considerable importance, and from purely theoretical considerations it might, perhaps, be expected to occur. We found, however, on continuing our experiments, that upon using a lighter and more sensitive membrane for recording pressure changes we were unable to reproduce our earlier records in this respect. In our later experiments the increase in electrical conductivity occurred at *approximately* the same time as the beginning of the rise in the intraventricular pressure. In one experiment the former occurred 0.05 second before the latter, in four at the same time, in the remainder from 0.01 to 0.05 second after. These differences we regard as within the limits of our error of measurement, and particularly of judging the exact moment of the beginning of the pressure rise.

The exact relation of the conductivity changes to the later dynamic events of systole is not entirely clear. We were particularly interested to note whether there was any correlation between the height of the pressure curve and the maximum change in conductivity. It is, of course, evident that in a general way increase in permeability is associated with systole, decrease with diastole and diastasis. But the peak of pressure did not coincide with the greatest conductivity change. In our earlier experiments the latter somewhat preceded the former. In our later ones, however, while this sometimes occurred, generally the maximum sound change occurred after the greatest rise in pressure. In interpreting this, we are confronted at once by a difficulty. As Wiggers (1925) has shown, the end of systole occurs after the peak of the pressure curve in mammalian hearts and bears no constant relation to it. Frank (1895) has made a similar observation in the frog's heart. Moreover, another important function of the heart, namely, its absolute refractory phase, which like the conductivity changes is also associated with systole, actually ends, as Wiggers has shown in the dog heart (loc. cit.) some time before the end of systole, and at a varying interval after the greatest rise in intraventricular pressure. We might expect, on purely theoretical grounds, that the refractory phase should bear a demonstrable relationship to changes in permeability. Unfortunately, the time relations between the peak of pressure and the absolute refractory phase in the tortoise heart have never been worked out. The actual significance of the summit of intraventricular pressure, in terms of the physico-chemical changes in the cardiac musculature, remain, therefore, obscure, and we prefer simply to state our findings without indulging in questionable speculation.

As to the relation of the conductivity changes to the end of diastole, the situation is clearer. During diastole there is a gradual increase in the electrical resistance of the heart. But this is not usually completed at the end of the relaxation period. During diastasis, when the heart is absolutely quiescent mechanically, the changes in conductivity continue for an appreciable interval. This is especially noticeable in those hearts in which

TA[illegible]

Changes in sound and their relation to pressure when the si[illegible]rt of the

EXPERIMENT NUMBER		TIME					
		0 second	0.5 second	1.0 second	1.5 seconds	2.0 sec[illegible]	2.5 second
1	Pressure	0	80	180	206	218=C	[illegible]
	Percentage of sound width	100	108	106	105	97	92.5
2	Pressure	0	144	188(C=1.04″)	178	140	90. E=2.95
	Percentage of sound width	100	124	119	110	111.5	107
3	Pressure	0	117	168(C=1.06″)	159	140	114
	Percentage of sound width	100	125	115	110.5	119	122.
4	Pressure	0	114	146=C	138	124	134
	Percentage of sound width	100	121	120	120	120.5	122.
5	Pressure	0	132(C=0.8″)	166	140	90	140
	Percentage of sound width	100	113	112.5	112	111.5	111
6	Pressure	0	100	134(C=1.25″)	134	120	94
	Percentage of sound width	100	98	112	133.5	142	12[illegible]
7	Pressure	0	68	104	115=C	108	94
	Percentage of sound width	100	144	174	208	224	22[illegible]
8	Pressure	0	100	130	138=C	132	118
	Percentage of sound width	100	101	103	128	142	141
	Average pressure	0	107	152	151	134	109
	Average percentage of sound width	100	115	120	128	133	132.

* C = the maximum height of intraventricular pressure. E = the end of diastole. F[illegible]re refer[illegible]

TA[illegible]

Changes in the sound and their relation to the pressure curve whe[illegible]ding cont

EXPERIMENT NUMBER		TIME					
		0 second	0.5 second	1.0 second	1.5 seconds	2.0 sec[illegible]	2.5 secon
2	Pressure	0	100	176	194(C=1.65)	189.5	179
	Percentage of sound width	100	91	84	84	87	88
3	Pressure	0	147	185.5=C	160	148.5	105
	Percentage of sound width	100	71	81	84	80	75
4	Pressure	0	126.5(C=0.85)	156	137	117.5	90.5
	Percentage of sound width	100	79.5	79.5	79.5	79.5	79.5
5	Pressure	0	155(C=0.83)	187	156	60	0=E
	Percentage of sound width	100	73.5	72	79.5	76	77.5
6	Pressure	0	123	150=C	112.5	62	0 E=3.2
	Percentage of sound width	100	85	73.5	63	57.5	61
7	Pressure	0	84	111=C	97	78	58
	Percentage of sound width	100	71	54	46	45	43

...t of the bridge is set to the left of the zero for diastasis

	TIME						
	2.5 seconds	3.0 seconds	3.5 seconds	4.0 seconds	4.5 seconds	5.0 seconds	6.0 seconds
218+5	[illegible]	140	28 (E=3.9")	0	0	0	0
97	[illegible]	93	99	99	99	99	99
140	0 (E=2.95")	0	0	0	0	0	0
113.5	[illegible]	100	100	100	100	100	100
140	[illegible]	54	8(E=3.64")	0	0	0	0
119	[illegible]	115	107	101	99	98.5	98
124	[illegible]	80	36	4(E=4.08")	0	0	0
120.5	[illegible]	120.5	109	104	102	101	100
90	[illegible]	2(E=3.04")	0	0	0	0	0
111.5	[illegible]	109	106	105	103	102	100
120	[illegible]	62	16(E=3.88")	0	0	0	0
142	[illegible]	122	113	111	109	106	103
108	[illegible]	74	52	15(E=4.44")	0	0	0
224	[illegible]	231	233	184	152	130	118
132	[illegible]	102	81	48	10(E=4.95")	0	0
142	[illegible]	133	130	122	113	106	104
134	[illegible]	64.5	27.5	8	1	0	0
133	[illegible]	128	122	116	111	106	103

...stole. ...re refers to the height of the pressure line in millimeters.

...curve when ...ing contact of the bridge is to the right of the zero for diastasis

	TIME						
	2.5 seconds	3.0 seconds	3.5 seconds	4.0 seconds	4.5 seconds	5.0 seconds	6.0 seconds
189.5	[illegible]	160	96	45	9(E=4.8)	0	0
87	[illegible]	87	88	92	94	95	97
148.5	[illegible]	22	0=E				
80	[illegible]	74	87.5	89	90	92	96
117.5	[illegible]	51	7(E=3.78)	0	0	0	0
79.5	[illegible]	87	96	97.5	98	99	
98	0=E	0	0	0	0	0	0
76	[illegible]	93	99.5	102	102	102.5	103
62	0(E=3.3)	0	0	0	0	0	0
57.5	[illegible]	64	75	84	88.5	91	92
78	[illegible]	23	2(E=3.7)	0	0	0	0
45	[illegible]	51	72	80	85.5	90	100

the beat was sufficiently slow to permit a relatively long period of diastasis (see curves Nos. 5 and 6). In practically all cases there is a continuing decrease in the permeability of the heart, which sometimes does not reach the base line until the onset of the next contraction. The apparent secondary rise in conductivity occurring in late diastasis and receding before the beginning of the next beat, which we observed in our preliminary observations, was present only inconstantly in our later experiments. It was always slight, and practically unmeasurable. We have, therefore, considered it advisable to disregard it, as we are unable to rule out to our satisfaction the possibility of artefact.

It should be stated, in passing, that the character of the conductivity changes was the same whether the stopcocks were turned (making the contraction isometric) during systole or during diastole.

II. Controls. In the discussion of technique, we have stated our method of making the contraction isometric. The only change in the volume of the ventricular cavity, upon closing the stopcocks, was the infinitesimal one associated with the movement of the rubber membrane by which we recorded pressure changes. It is clear that changes in the volume of the heart—hence changes in the thickness of the ventricular wall—might produce changes in the electrical conductivity. We record (fig. 4, *1*) the effect of artificially changing the volume of the heart by alternately raising and lowering a reservoir of saline connected with the ventricle. In the photograph the lower line indicates the changes in pressure that resulted from this method of changing the volume. With the augmentation in volume (denoted by the upstroke in the pressure curve) there is an increase in conductivity, due to thinning of the ventricular wall; with a diminution in volume there is a decrease in conductivity. Even if the contraction, in our experiments, had not been completely isometric, we would expect the error to be in the opposite direction to the results which we actually obtained, for the thickening of the wall associated with systole would have tended to cause a decrease in the electrical conductivity.

In making the observations on the isometric contraction, it was observed that there was frequently a certain amount of "twisting" of the ventricle. This twisting was sometimes obvious, sometimes barely visible. It remained, however, as a possibility of artefact. We therefore twisted the heart to a degree far more violent than any twisting observed in our normally beating hearts, by manipulating the cannulae inserted into the ventricle. It will be seen in figure 4, *2*, that this treatment did not cause appreciable changes in the sound. In the record shown two normal beats supervened, and were accompanied by the usual conductivity changes. This artificial twisting also ruled out another possible artefact, namely, the movement of fluid between the electrodes.

While the alternating current that we used may conceivably have had a stimulating effect on the heart, it was present equally throughout the cardiac cycle, and hence could not have been the cause of the phenomena we have discussed. It was possible, however, that the action current of the heart might have produced the unbalance of the Wheatstone bridge which we recorded as sound changes and interpreted as changes in the electrical resistance of the heart. We therefore shut off our artificial source of current, and recorded the effect of the heart current itself (fig. 4, *3*). No variation in the width of the sound record was demonstrable, hence this source of error could be ruled out. We are, therefore, justified in regard-

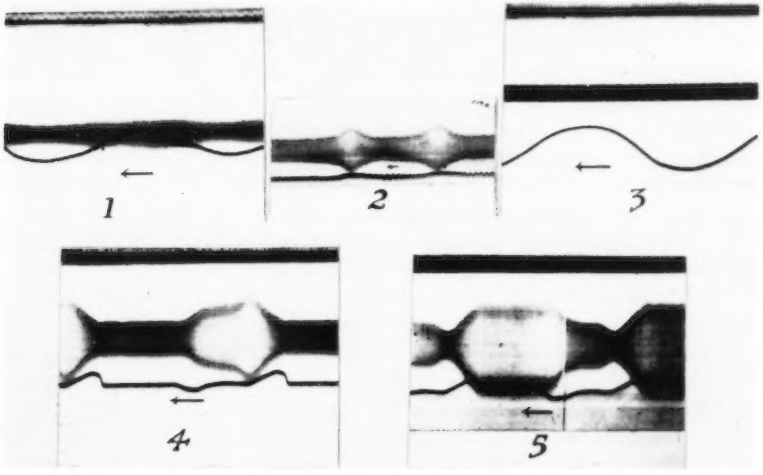

Fig. 4. *1*. Effect on sound width of artificially changing ventricular volume.
2. Effect on sound width of twisting the ventricle (two normal beats supervene).
3. Effect on sound width of shutting off source of current.
4 and *5*. Isotonic contractions (lower line in all curves represents intraventricular pressure).

ing the observed phenomena as being due to the physico-chemical changes peculiar to the contraction itself, resulting in variations in the permeability of the muscle.

III. The magnitude of the conductivity changes. We have already discussed our method of determining the directional character of the variations in conductivity. It is one of the limitations of the method that, in order to make quantitative estimations of the extent of the observed differences, we are obliged to compare at least two beats on different sides of the "zero point" on the slide wire. Unfortunately for such estimations no two beats are quite alike, nor does the condition of the heart during dias-

tasis remain the same. Hence the resistance of the heart at any point in a particular beat is difficult to determine with exactitude. It may be stated, however, that the difference between the minimum and maximum sound within a single beat represented a change of about 10 per cent of the total resistance of the heart.

IV. The isotonic contraction. The results which we obtained, as will be seen from the control observations, derived their significance from the fact that the observations were taken under isometric conditions. It has been noted in discussion of the control curve (fig. 4, *1*) that as the ventricular wall becomes thinner, the electrical resistance of the heart diminishes, as would be expected. This introduces an unknown variable into the conductivity changes observed during an isotonic contraction. We nevertheless made routine observations of the changes in the isotonic contraction in the course of our experiments. While in some of these observations there was evidence of an increase of conductivity during systole, in many others there was a decrease. This is not remarkable in view of the fact that the decrease in resistance due to the physico-chemical changes associated with the act of contraction *per se* was opposed by an augmentation of resistance due to the thickening of the wall as the volume decreased. The actual observed change would be a resultant of these factors; the direction of the change depending upon which of the two predominated. In figure 3, *4* and *5*, we show the record of such a contraction on both sides of the "zero point." In this particular experiment, there was a slight transient increase in permeability after onset of contraction. This is almost at once, however, succeeded by an abrupt decrease, followed in turn, during diastole, by a return to the initial state in apparently two distinct stages. The greatest thickening of that portion of the wall through which most of the current passed (it must be remembered that because of the diameter of the intraventricular cannula this was a comparatively small area) apparently occurred after the greatest rise in intraventricular pressure.

SUMMARY

1. A method is described for continuously recording changes in electrical conductivity.

2. It is found that associated with the systole of the tortoise ventricle beating isometrically, there is an increase in the permeability.

3. This increase commences at the same time (*within the limits of error of measurement*) as the beginning of the rise in intraventricular pressure.

4. The maximum increase in conductivity bears a variable relation to the peak of intraventricular pressure, occurring usually somewhat later, but occasionally shortly before the latter.

5. During diastole the conductivity decreases. This change continues in diastasis, when the heart is mechanically quiescent.

6. The greatest change in conductivity during a single beat is approximately 10 per cent of the total resistance of the ventricle.

7. Observations on the isotonic contraction are complicated by volume changes involving variations in the thickness of the ventricular wall.

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EFFECTS OF CARBON MONOXIDE INHALATION UPON METABOLISM

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Received for publication October 12, 1926

The literature of carbon monoxide, voluminous as it is, supplies very little information on the effects of this gas upon metabolism, especially when inhaled in low concentrations with the air. As discovered by Claude Bernard in 1857 (1), carbon monoxide possesses the property of forming a definite compound with the hemoglobin in the blood stream and in this way blocking the passage of oxygen to the cells. As shown by Haldane in 1895 (2) and by Nicloux (3) and others later, the reaction between carbon monoxide and hemoglobin is reversible and subject to the law of mass action. Hence in the absence of free carbon monoxide, the hemoglobin compound decomposes, permitting the blood to free itself of the gas and regain its oxygen carrying capacity. Aside from these reactions, Haldane (2), Haggard (4) and others have shown carbon monoxide to be physiologically an inert gas without toxic effects upon the tissues. These facts suggest the conclusion that so-called carbon monoxide poisoning is in reality an anoxemia within the cells which in turn *interferes with cell metabolism*, though experimental evidence supporting this latter view is lacking, as stated above.

The purpose of this research was to test the validity of the second part of this conclusion. The specific problem attacked was that of determining what effects upon *the rate* of an animal's metabolism might result from the breathing of carbon monoxide in controlled concentrations in air.

Experimental conditions. The animals used in the research were chiefly white rats. Canary birds were used to some extent, but mainly to confirm results obtained with the rats. Both the rats and the birds were kept on a uniform diet during the progress of the experiments—the birds upon a mixture of hemp and rape seeds and the rats upon a balanced diet prepared according to McCollum's formula no. 211 (wheat 67.5, casein 15.0, whole milk powder 10.0, NaCl 1.0, $CaCO_3$ 1.5, and butter fat 5.0 per cent respectively). During the time of taking the metabolism the animals were kept as nearly as possible in the same degree of activity. Efforts were also made to habituate them to the processes involved so that the element of fright might be eliminated.

In exposing the animals to carbon monoxide, jars of the type shown in figure 1 were used, these having a capacity of 7.4 liters each. The aim was to supply an atmosphere in all respects normal, except for the carbon monoxide, but to offset possible defects in the arrangement, the air was completely changed at periods of from one to three hours. To provide some circulation within the jars and also to prevent a rise of temperature from the animal's heat, cloths wet with cold water were kept on the back ends. When this alone was not sufficient to keep down the temperature, a current of air from an electric fan was directed upon the wet cloth. Room temperature was maintained within the jars as well as in the small chambers, referred to later, in which the animals were placed for determining their metabolic rates.

Technic of carbon monoxide administration. The procedure in exposing the animal to a definite carbon monoxide concentration was first to place it in the jar described above and tightly close the stopper. A 100 cc. syringe was then connected with the entrance tube (*3*, fig. 1) after which the charge of carbon monoxide was introduced through the side of this tube by means of a hypodermic needle and syringe. From here the gas was forced into the jar with the 100 cc. syringe which was then filled from and emptied into the jar several times to insure rapid mixing. After this, connection was made with an oxygen bag as indicated in figure 1.

The carbon monoxide used in the experiments was generated by the action of concentrated sulphuric acid on sodium formate and stored under pressure in gas collecting bottles. For filling the small syringe preparatory to placing a charge of carbon monoxide in the exposing chamber, a rubber tube from the storage bottle was connected with a pointed glass tube terminating in an open vessel under water. The syringe was filled by puncturing this tube with the hypodermic needle. To prevent escape of gas in transferring the charge to the exposing chamber, the needle was kept pointing downward, the body of the syringe protected from the heat of the hand, and the piston kept lubricated. The purity of the gas was determined by the Haldane air analysis apparatus (3). This determination was made as soon as the gas was generated and thereafter whenever there was any doubt as to its condition.

Determination of the metabolic rate. The method of carbon dioxide production was used for this purpose. This required for animals as small as birds and rats, an accurate method of determining the amount of carbon dioxide produced and such an arrangement of the apparatus that the time of collecting the carbon dioxide could be accurately measured. This method also required an efficient agent for removing moisture from the air before the carbon dioxide was absorbed and also for taking up any moisture that might result from the union of the carbon dioxide with its absorbent.

With our present knowledge of apparatus and chemicals for making

carbon dioxide and moisture determinations, the securing of suitable tubes for this purpose would seem a very simple matter. In actual practice much difficulty was encountered. Cumbrous U-tubes were not adapted to small balances and the standard moisture absorbents, such as calcuim

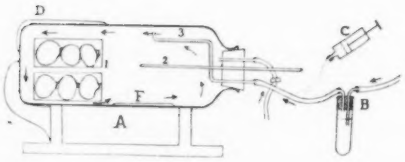

Fig. 1

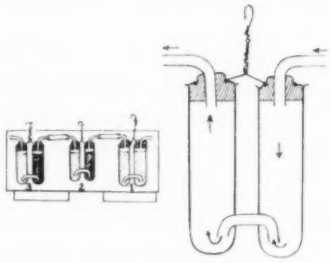

Fig. 2

Fig. 1. Apparatus for exposing small animals to different concentrations of carbon monoxide in air.

A. Large jar mounted on wooden support in which the animal is placed. This has a tier of wire cartridges at the back, *1*, which are filled with bags of moist soda lime for absorbing carbon dioxide, a thermometer, *2*, which is read through the glass from the outside, and a bent tube, *3*, for the introduction of carbon monoxide and oxygen, at different times, from the outside.

B. A water valve which is on the line to an oxygen bag not shown in the illustration. As the carbon dioxide is absorbed by the soda lime, oxygen is drawn from the bag to replace that which is consumed by the animal.

C. Syringe for forcing in the charge of carbon monoxide (introduced at the arrow nearest the jar) and for mixing it with the contained air. This is connected only during the time of putting in the carbon monoxide, replacing *B*.

D. Wet cloth upon which an electric fan plays as explained in the text.

Fig. 2. Special absorption tube designed for this investigation.

In principle this is a modified U-tube. It is adapted to the use of two kinds of solid absorbents at the same time but, like the regular U-tube, can have the same absorbent on both sides. For small animal determinations, the inside diameters should be one inch and the length about 4.5 inches. The cork stoppers, made flush with the glass at the edges, are raised at the center and grooved to receive the small copper wires by which the tube is suspended. The apparatus is not fragile as might be supposed.

On the left is shown three of these tubes in series as used in this investigation. Both arms of tube *1* are filled with pumice granules (8 to 12 mesh) covered with phosphorus pentoxide powder. This tube takes up moisture only. Tube *2* has soda lime in the right arm and pumice and phosphorus pentoxide powder in the left and absorbs both carbon dioxide and moisture. Tube *3* is a duplicate of tube 2 and is used as a check on its efficiency. After filling, the stoppers are sealed with paraffine.

chloride and concentrated sulphuric acid, were not satisfactory. Soda lime for taking up the carbon dioxide also gave trouble, that supplied by one manufacturer being too dry for the purpose and that supplied by another being too moist. The problem was finally solved by the invention

of the special absorption tube shown in figure 2, the discovery of a practical method of using phosphorus pentoxide powder as a moisture absorbent (covering pumice granules with the powder by shaking in a closed bottle), and the finding of the right proportions in which to mix the dry soda lime with the moist soda lime, for the best results in taking up the carbon dioxide.

These absorption tubes proved to be all that had been hoped for them. They were adapted to quick and accurate weighing, were easily replenished and lasted well when filled, and in the absorption of carbon dioxide and moisture from air passed rapidly through them (one to two liters per minute), were almost 100 per cent efficient. In addition to this, the degree to which they were exhausted could be easily noted. Pumice covered with phosphorus pentoxide powder is white, but when saturated with moisture, the mixture becomes gray and looks wet. This change shows at a glance how nearly the pentoxide is spent. Exhaustion of the soda lime in tube *2* is shown by an increase in weight of tube *3* (fig. 2), provided the pentoxide in tube *2* is not exhausted. After some experimenting, the plan was adopted of renewing the contents of the right arm of tube *1* as soon as the moisture had reached the bottom and of changing the soda lime in tube *2* when tube *3* began to increase in weight and the moisture was still below the middle of the left arm in tube *2*.

In determining the rate of metabolism from the amount of carbon dioxide produced, the animal was placed in a small, tightly closed chamber through which air was drawn by the laboratory vacuum system, this being connected with a meter and pressure gauge by which the rate of flow was measured. Before entering the chamber the air was deprived of all of its carbon dioxide by passing through a tower of soda lime. From the chamber the air tube was divided, one branch connecting directly with the meter and the other passing to the absorption tubes described above and then through the meter into the vacuum system, the flow either way being controlled by cocks.

On putting the animal into the metabolism chamber, the air was all passed directly through the meter into the vacuum system, but the instant the determination was to begin, the air from the chamber was thrown through the absorption tubes. No further adjustment was then made until the time for the determination had expired (5 to 20 minutes depending on the size of the animal) when the air was quickly turned in the other direction.

The carmine test for carbon monoxide. For making determinations of carbon monoxide in air and in blood, Haldane's standard carmine method was chiefly used. This is adapted to laboratory procedure, is fairly accurate and delicate, and can be used for testing both air and blood. It was found, however, that to give consistent results, the method had to be very care-

fully carried out. As explained by Haldane in his small book on *Air Analysis* and later in his work on *Respiration* (5), the blood solution must be accurately prepared, the carmine solution definitely standardized, and the carbon monoxide blood protected from strong light. In preparing the blood solution for testing carbon monoxide in air, the plan was followed of first making a hemoglobinometer test. The blood and water were then proportioned to give the desired percentage of hemoglobin. For example, if the sample of blood showed 85 per cent by the hemoglobinometer test, the volume of blood taken was $\frac{0.5}{0.85}$, or 0.59 per cent, of the volume of the water used.

To guard against the dissociating effect of strong light, the top of the table on which the tests were made was enclosed on three sides with beaver board painted black, and for matching colors a 75 Watt daylight Madza lamp was placed in a ten inch square metal box having frosted glass on one side. A jar of water between the lamp and the glass absorbed most of the heat and a small adjustable curtain of white cloth protected the eyes of the observer. This supplied a light of uniform intensity which was equal to sunlight in giving color contrasts and was ready for use at any time. The convenient pyrotannic acid method of Sayers, Yant and Jones (6) was used to some extent in verifying the tests made by the carmine method.

Preliminary determinations. Before starting the experiments with the carbon monoxide, a series of metabolic tests was made to see what the normal range of rats and birds was under different conditions and to determine in particular the effects upon metabolism of confinement for different periods in the exposing chamber (fig. 1). The rates shown in tables 1 and 2 are typical of the results obtained.

The most important conclusion reached from these experiments was that the rate of metabolism was greatly increased by activity. The rate was slightly increased from the taking of food, but confinement for three hours in the exposing chamber had little or no effect. The rates in italics were determined when the rat was asleep. They show the effect of diminished activity and bring out the fact that during sleep, as well as in the waking period, the rate of metabolism is fluctuating.

The cautious administration of carbon monoxide was now begun, starting with concentrations of 0.01 per cent and trying successive mixtures with air up to 0.05 per cent. No significant change in the rate of metabolism of the bird was observed, but with the rat a decided fall in the rate was obtained with 0.05 per cent. This is shown in table 3.

Not only are the metabolic rates at the close of the last two periods with 0.05 per cent abnormally low for an animal awake, as in this case, but they are lower than the sleeping metabolisms shown in table 2 for the same rat. Distinct symptoms of carbon monoxide poisoning were also

shown, the rat being very weak and sluggish. A drop of blood obtained by clipping the tail showed, when diluted, a distinct carmine tint.

It was hoped, however, that effects upon the rate of metabolism would appear with concentrations of carbon monoxide below 0.05 per cent. Since

TABLE 1

Metabolism of bird 1 (cc. of carbon dioxide per gram per minute) after definite periods of confinement in the exposing chamber

DATE	AT CLOSE FIRST HOUR	CLOSE SECOND HOUR	CLOSE THIRD HOUR
January 27	0.0676	0.0659	0.0676
January 29	0.0653	0.0680	0.0504
February 1	0.0727	0.0711	0.0700
February 3	0.0628	0.0700	0.0640
February 5	0.0681	0.0720	0.0670
Average	0.0673	0.0694	0.0638

TABLE 2

Metabolism of rat 1 after definite periods of confinement in the exposing chamber

DATE	AT CLOSE FIRST HOUR	CLOSE SECOND HOUR	CLOSE THIRD HOUR
January 28	0.0261	0.0258	0.0237
January 30	0.0263	0.0339	*0.0142*
February 2	0.0264	0.0263	0.0229
February 4	0.0273	*0.0184*	0.0277
February 6	0.0304	0.0205	*0.0176*
Average	0.0273	0.0266	0.0244

TABLE 3

Metabolism of rat 1 after definite periods of confinement with varying percentages of carbon monoxide in air

DATE	AT CLOSE FIRST HOUR	CLOSE SECOND HOUR	CLOSE THIRD HOUR	PER CENT OF CO USED
February 9	0.0307	0.0270	0.0259	0.01
February 11	0.0241	0.0188	0.0178	0.02
February 16	0.0298	0.0283	0.0214	0.03
February 20	0.0285	0.0256	0.0207	0.04
February 22	0.0255	0.0131	0.0129	0.05

using the same animal for all the tests with carbon monoxide gave opportunity for acclimatization, it was decided to repeat the tests for the concentrations above 0.02 per cent, using a fresh rat each time and also making control experiments. Not only was there failure to find evidence of effects below 0.05 per cent, but the results were such as to throw doubt

upon the previous results for 0.05 per cent. To make sure that a positive effect upon metabolism would eventually be obtained, two rats were exposed for three hours each to 0.08 and 0.10 per cent respectively. Their metabolic rates taken at the beginning of the experiment and then at the close of each hour period, were as follows:

a. Metabolic rate of rat 9 with 0.08 per cent of carbon monoxide

BEFORE ADMINISTERING THE CO	AT CLOSE FIRST HOUR PERIOD	CLOSE SECOND HOUR PERIOD	CLOSE THIRD HOUR PERIOD
0.0197	0.0127	0.0122	0.0122

b. Metabolic rate of rat 8 with 0.10 per cent of carbon monoxide

BEFORE ADMINISTERING THE CO	AT CLOSE FIRST HOUR PERIOD	CLOSE SECOND HOUR PERIOD	CLOSE THIRD HOUR PERIOD
0.0149	0.0115	0.0112	0.0092

There was now no longer any doubt as to the depressing effect of carbon monoxide upon the rate of the animal's metabolism when a sufficient amount of it was breathed. It only remained to determine at what concentration the depression began and to relate this, if possible, with the accumulation of carbon monoxide in the blood.

Fall in body temperature an index to depressed metabolic rate. The last two determinations, in addition to supplying positive proof of the depressing effect of carbon monoxide upon the animal's metabolism, also suggested an additional index to the degree of the depression—that of a fall in body temperature. Both rats at the close of their exposures were cold to the touch, the tails and ears especially so. While their temperatures could not be taken at the time, it was decided to make temperature tests a part of the routine procedure for the future. Rectal temperatures were accordingly taken of several rats to get their normal range and to see how the bulb should be inserted to get true readings. Their temperatures ranged from 100 to 101.8°F. Later in testing the effects of carbon monoxide when the temperatures were too low for the clinical thermometer which did not register below 92°F., the ordinary chemical thermometer was used and the Centigrade readings converted to Fahrenheit readings. In obtaining the temperatures which follow, care was taken to first remove any feces that had collected in the rectum, to insert the thermometer bulb at least 1.5 inches, and to expose the "minute" instrument for a minute and a half.

Effects of high concentrations. A series of experiments was now made to determine how far the rate of metabolism could be depressed and the temperature lowered without seriously injuring the animal. Beginning with a concentration of 0.08 per cent, this was increased on successive days, the amount administered being determined by the reactions at the lower con-

centrations. Fresh rats were used in each case, the subject being killed at the close of the experiment in order to get blood for testing the degree of saturation. (Both the carmine and the pyrotannic acid tests were made.) The results of these experiments are shown in table 4.

None of the rats died as a result of the carbon monoxide exposure. In fact the experiment could have been carried considerably further than it was without causing death, as the stage of coma was in no instance reached. Both in the above experiments and in those that follow, marked individual differences were manifest. The healthy and vigorous rats were much more

TABLE 4

Effects upon the metabolic rate and the body temperature from high concentrations of carbon monoxide and long exposures

RAT USED	TIME EXPOSED	PER CENT OF CO	METABOLISM AT START	METABOLISM AT CLOSE	PER CENT OF FALL	BODY TEMPERATURE AT CLOSE	PER CENT OF SATURATION OF THE BLOOD
18	5 hours	0.08	0.0322	0.0129	60	93.2°F.	46
13	3.0	0.10	0.0238	0.0181	24	97.3	25
16	4.0	0.10	0.0246	0.0127	48	94.3	46
14	3.5	0.15	0.0225	0.0124	44	94.3	46
15	3.5	0.20	0.0218	0.0087	60	87.8	55
17	4.5	0.25	0.0212	0.0073	65	82.4	66

TABLE 5

Effects of repeated exposures to carbon monoxide in concentrations ranging from 0.06 to 0.08 per cent for five successive days

	RAT 19. AFTER 2 HOURS EXPOSURE TO 0.08 PER CENT		RAT 20. AFTER 2 HOURS EXPOSURE TO 0.08 PER CENT		RAT 21. AFTER 2 HOURS EXPOSURE TO 0.08 PER CENT	
	Metabolism	Body temperature	Metabolism	Body temperature	Metabolism	Body temperature
First exposure.....	0.0224	95.0°F.	0.0203	98.2°F.	0.0246	98.5°F.
After 5 days of exposures	0.0283	97.7	0.0253	99.8	0.0253	99.6

tolerant of the gas than were the rats whose physical condition was in some respects below normal.

The principle of compensation. That a process of compensation takes place when an animal is exposed for some time to non-lethal concentrations of carbon monoxide was proved in the case of rats, as follows: Three rats were first exposed for two hours to 0.08 per cent of carbon monoxide after which their metabolic rates and temperatures were taken. They were then exposed six hours per day for five successive days to atmospheres ranging from 0.06 to 0.08 per cent carbon monoxide. On the day following the last exposure of the series, they were again exposed for two hours to a concentra-

tion of 0.08 per cent and their metabolic rates and temperatures taken. The results are shown in table 5.

These results show the general trend of effects and are in accord with the findings of Nasmith and Graham for guinea pigs (7). Each rat showed a greater tolerance for carbon monoxide after the five days of exposures.

Effects of long exposures with low concentrations. A series of long exposures was now begun with the low concentrations with which there had previously been repeated failure to get positive results—those from 0.01 to 0.04 per cent. The rats after having their metabolic rates taken were placed in the exposing chambers and kept with the desired percentage of carbon monoxide for three hours. The air was then completely changed and the carbon monoxide renewed, after which they were again exposed for three hours. They were then removed and their metabolic rates and temperatures taken. The aim was to give plenty of time for the blood to become saturated to the fullest extent possible for the concentration used.

TABLE 6

Effect on metabolic rate and body temperature from 6 hours' exposure to 0.02 per cent of carbon monoxide in air

RAT USED	METABOLISM AT BEGINNING	METABOLISM AT CLOSE	BODY TEMPERATURE AT CLOSE	GENERAL REACTION
25 (subject)	0.0262	0.0220	98.5°F.	Positive
26 (subject)	0.0244	0.0281	101.8	Negative
29 (subject)	0.0304	0.0207	96.6	Positive
36 (subject)	0.0296	0.0247	99.6	Doubtful
37 (subject)	0.0212	0.0237	99.5	Negative
38 (subject)	0.0270	0.0223	96.0	Positive
31 (control)	0.0253	0.0272	101.8	

Positive results were obtained in individual cases for 0.04, 0.03 and 0.02 per cent. The results for 0.02 per cent are shown in table 6. Only negative results were obtained with the four rats exposed to a concentration of 0.01 per cent as above.

Correlation of the depression in metabolic rate with the symptom of carbon monoxide poisoning. In all of these experiments the depression in the rate of metabolism and fall in body temperature corresponded with the degree to which symptoms of carbon monoxide poisoning (chiefly weakness and unsteadiness) were manifested. The more pronounced the symptoms the greater the depression in the rate and the greater the fall in temperature. This suggested a correlation between the symptoms and the depression of the metabolic rate. Henderson and Haggard have shown (8) that marked and rather severe symptoms occur in human beings when the blood reaches a saturation of from 27 to 35 per cent and the Bureau of Mines, continuing these investigations (9), has shown that mild subjective

symptoms result from six hour exposures to 0.02 per cent, this causing a blood saturation of from 16 to 20 per cent. With all of these saturations a fall in the rate of metabolism was obtained with the rats.

CONCLUSIONS

1. Carbon monoxide depresses the rate of metabolism, the degree of depression varying directly with the degree of saturation.

2. There is a lowering of body temperature that corresponds closely with the slowing of the metabolic rate.

3. The breathing of carbon monoxide concentrations of from 0.02 to 0.05 per cent for short periods (1 to 2 hours) has little if any effect upon the rate of metabolism of the rat, but for long periods (4 to 6 hours) it depresses the rate in individual cases.

4. Concentrations above 0.05 per cent have marked effects in depressing the rate even for short periods of exposure.

5. There is a correlation between the fall in the metabolic rate and the symptoms of carbon monoxide poisoning.

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THE EFFECT OF UNILATERAL LABYRINTHECTOMY ON ROTATIONAL NYSTAGMUS

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Received for publication October 13, 1926

In a paper dealing with the effects of extirpation of the embryonic ear on equilibrium in Amblystoma, Greene and Laurens (1923) reported observations indicating that although each labyrinth influences the eyeball rotations of both eyes and when one is removed there is still binocular movement, there is evidence that the influence of the remaining labyrinth is greater on one eye than on the other. The evidence is the fact that in some cases when the direction of rotation was to the operated side the eye deviation was slighter than when it was toward the normal side, and that the most pronounced post-rotatory effect was observed when the rotation had been toward the operated side. It was further found, when the influence of moving retinal images was excluded by surrounding the animal during rotation by an opaque cylinder, that when the direction of rotation was toward the operated side the compensatory rotatory reaction was slight or absent, while the post-rotatory was marked; and that when the direction of rotation was toward the normal side, the rotatory reaction was large, though not as great as when the opaque wall was not present, and the post-rotatory slight or absent. Although the influence of the retina was thus demonstrated the movements of the eyes seemed to be chiefly controlled by the labyrinths, and it was only in some instances when the animal was surrounded by an opaque cylinder that the rotatory movements diminished or disappeared while the post-rotatory increased. Ever since the demonstration of these facts in Amblystoma, one of us (H. L.) has wished to see whether anything of the sort could be demonstrated in a mammal and the work here reported on was started in the fall of 1922.

[1] The results here reported are taken from the essay presented by Dr. A. L. Miles to the Faculty of the Graduate School of Yale University in candidacy for the Degree of Master of Science in June, 1924. Their publication has been delayed for various reasons, chiefly in the hope that the observations on the animal with head held vertically might be extended. The possibility of doing this being remote, it has been decided to delay no longer the publication of the results already obtained.

Pilz (1926) has recently shown that there is a reciprocal relation between rotatory nystagmus in one direction of rotation and post-rotatory nystagmus in the other in the majority of normal rabbits, as well as in those with unilateral cerebral or labyrinth injury. Following the destruction of one labyrinth rotatory nystagmus was less upon rotation toward the injured side than upon rotation toward the intact side while post-rotatory nystagmus was greater following rotation to the injured side than to the intact side.

Schiff (1891) reported that in the frog and the dog there is no reaction upon rotation toward the injured side. Ewald (1892), however, found in the frog that rotatory and post-rotatory reactions occur during and after rotation in both directions, but much weakened, especially during rotation to the operated side and after rotation toward the sound side. Trendelenburg and Kühn (1908) observed a differential influence following unilateral labyrinthectomy in a lizard and in a mud turtle. When rotated toward the side with the lesion, the eyes being open, both rotatory and post-rotatory compensatory movements occurred. Bartels' (1910) results indicate that the destruction of one labyrinth markedly diminishes rotatory nystagmus during rotation toward the injured side and post-rotatory nystagmus following rotation toward the intact side. Prince (1917) noted in unilaterally labyrinthectomized young cats, tied to a turn table with the vertex up, that the deviation of the eyes was intensified upon cessation of rotation when this had been toward the side of the lesion, but entirely disappeared when the direction of rotation had been toward the intact side. De Barenne and deKleijn (1923) state that with right labyrinthectomy a greater post-rotatory nystagmus is obtained after rotation to the right than after rotation to the left. However, in many normal rabbits there is a slight difference in post-rotatory nystagmus following rotation to the two sides. Masuda (1922) reports similar findings in guinea pigs. Detlefsen (1925) found in rats with one injured labyrinth that post-rotatory nystagmus following rotation toward the injured side is greater than that following rotation toward the sound side.

The evidence that the retina had anything to do with rotatory and post-rotatory compensatory reactions was first clearly set forth by Loeb, who separated the compensatory motions of eyes and head caused by the orienting effect of moving retinal images from those caused by the orienting effect of centrifugal force. He showed that when a lizard, Phyronosoma, is rotated very slowly with its eyes closed only very slight compensatory motions of the head and body are observed during rotation, while very powerful compensatory motions are produced when the motion of the turn table is suddenly interrupted. With the eyes open the reverse is obtained, the compensatory motions during rotation being vigorous while the post-rotatory are slight. When the eyes are closed there is only

the geotropic effect of passive rotation; when the eyes are open the orienting influence of moving retinal images is added algebraically to the orienting effect of centrifugal force. These two influences act in the same sense during the rotation and therefore are additive, while after rotation they act in the opposite sense to each other.

This observation has been found to hold in a number of forms, both normal and with one ear removed. Trendelenburg and Kühn (1908) found in unilaterally labyrinthectomized lizards and turtles that rotation to the injured side with the eyes closed produced no compensatory but marked post-rotatory movement, and rotation to the sound side rotatory compensatory movement but no post-rotatory. Maxwell and Pilz (1924) showed that retinal modification of labyrinthine excitation occurs in the rabbit, as exemplified in the greater reduction in rotatory nystagmus in light than in darkness following repeated rotation. Pilz (1926) also demonstrated that labyrinthine post-rotatory nystagmus can be modified by retinal stimulation. Taking alternate records of unhooded and hooded animals he found that in the majority of cases there was less rotatory and more post-rotatory nystagmus when the eyes were covered than when they were not. When similar observations were made on unilaterally labyrinthectomized animals it was found that both rotatory and post-rotatory nystagmus still occurred from which he concluded that the residual rotatory nystagmus occurring after rotation toward the injured side and the post-rotatory nystagmus toward the opposite side are not optical in character. Greene and Laurens' results were given above.

The rotation apparatus was a round table fastened securely to the drum of a Kershaw kymograph. The power was transmitted by a belt from a small motor and the speed of rotation was controlled by using different sized pulleys on the driving shaft of the kymograph, and by throwing in or out resistance. The direction of rotation was changed by crossing the belt. In certain cases the rotation was done by hand.

Results on the frog. *Compensatory reactions of normal frogs.* A few preliminary tests were made on normal frogs. Unless the speed is low the eye movements are obscured by the vigorous head movements. The difference between the results of rotation when moving retinal images play a part and when their action is excluded are easily demonstrated. With moving retinal images the compensatory movements during rotation are numerous and vigorous, while the post-rotatory are comparatively few and weak. The rate of rotatory nystagmus was about 5 in 10 seconds for a speed of rotation of one complete revolution in 10 seconds. After two or three trials the frog becomes fatigued so that only a few observations can be made on an individual at any one time. When the influence of moving retinal images is excluded by surrounding the frog by an opaque jar rotatory nystagmus is less frequent and weak, while post-rotatory nystagmus is marked and vigorous.

Compensatory reactions of labyrinthectomized frogs. After the reactions of a number of normal frogs had been observed the labyrinth on one or both sides was destroyed. When bilaterally operated animals are rotated so that the visual field moves, both rotatory and post-rotatory nystagmus are observed, when the visual field is stationary neither can be elicited. When unilaterally labyrinthectomized frogs are rotated, rotatory nystagmus is obtained upon rotation in either direction, but the post-rotatory reaction is difficult to demonstrate. The possibility arose that rotatory nystagmus might in some cases be due to the stimulation caused by moving retinal images. Their action was therefore excluded, and it was found, in the large majority of cases, that when the direction of rotation is to the side from which the labyrinth has been removed no rotatory nystagmus is observable. Only occasionally were one or two quick movements of the eye seen. But following rotation to the injured side post-rotatory nystagmus is especially marked and vigorous, four to five movements being the average. Furthermore, when the animal is rotated toward the normal side, strong rotatory, but no or little post-rotatory, nystagmus is obtained. For further proof of this differential action the optic nerves were cut in a frog, after which the right labyrinth was removed. When this frog was rotated toward the normal side rotatory, but no post-rotatory, nystagmus occurred; and when rotated toward the injured side post-rotatory nystagmus only.

Decerebrate frogs react like normals to rotation and decerebrate frogs with one labyrinth injured give the reaction typical of unilaterally labyrinthectomized frogs.

Results on the rabbit. The rabbit was placed in a box fastened to a board on the rotation table. One side of the box was movable, so that its width could be varied to accord with the size of the rabbit. Cloths and cotton wool packed around the animal held it securely and comfortably. The rabbits were observed during and after rotation with the head free to move, and with it held securely horizontally as well as vertically with the occiput up and down respectively. Only the first two sets of results will be considered.

Compensatory reactions of unilaterally labyrinthectomized rabbits to rotation. Having obtained a clear conception of the compensatory eye movements of normal rabbits to rotation, the study of the effects of unilateral labyrinthectomy was taken up. The operation for destruction of the labyrinth was in all essentials that of Magnus and deKleijn (1913), and need not be described. The reactions of the animal leave no doubt as to whether the operation has been a success, and the characteristic postural incoördination is evident even before the animal recovers from the morphia. The condition and reactions of the animal immediately after the operation have been adequately described by Magnus and de-

Kleijn (1913) the most notable effects being the violent rolling movements toward the operated side, the bending and torsion of the head, and the deviation and nystagmus of the eyes. Although operative nystagmus gradually decreases and in most animals disappears within twenty-four to forty-eight hours, it returns for a short time if the animal is seriously disturbed.

Five rabbits were successfully operated on, that is to say, they exhibited after operation typical disturbances of posture. They were therefore subjected to observation during and after rotation. Slow and fast rotation by motor and very slow rotation by hand was carried out. The slow

TABLE 1

Head held horizontally; showing the differential effects of rotation toward the intact and injured side of unilaterally labyrinthectomized rabbits

	NUMBER OF ANIMAL																
	1		2	3				4				5					
	March 12	March 14	April 6	April 20	April 21	April 23	April 25	April 30	May 2	May 4	May 7	May 15	May 18	May 18†	May 21	May 21†	May 25
Rotation toward intact side																	
Slow rotation once in 5 seconds			+	−	+	−	+	+	+	+		+	+	?	+	?	
After slow rotation	*		*	−	?	+	−	*	+	−	−	?	+	?	−	−	
Fast rotation once in 2 seconds			+	−	+	−		+	+			+		?	+	?	+
After fast rotation		−	?	?	?	−	?	+	−	?	−	−	+	−	?	?	?
Rotation toward injured side																	
Slow rotation once in 5 seconds	−	−	+	−	−	−	−	?	−			?	?	−	−	−	
After slow rotation	+	+	+	+	?	?	+	+	+	+	+	+	+	+	+	+	
Fast rotation once in 2 seconds			−		−	−	−	−	−	−	−	+		−	?	?	−
After fast rotation		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

† Blindfolded.

+ = nystagmus; ? = slight nystagmus; − = no nystagmus; * = operative nystagmus.

speed was one revolution in five, the fast one revolution in two seconds for a total of 10 revolutions.

Observations of this sort made on the eyes of an animal with the head free to move are affected by the head movements. The rotatory compensatory position assumed by the head makes the nystagmus less than when the head is turned the opposite way, the effect of the head position thus partially neutralizing the reflex from the internal ear, and the actual result is the algebraic sum of two quantities of opposite sign. The results with the head held horizontally are shown in the table. In the majority of cases during both slow and fast rotation toward the intact side there is

strong compensatory deviation and nystagmus, the operative and rotatory deviation being in the same direction, but the post-rotatory response is weak or absent. Rotation toward the operated side is accompanied by but slight or no compensatory movements, operative and rotational nystagmus having opposite tendencies, but the post-rotatory movements are very striking.

One of the animals, no. 5, was observed under conditions which excluded the action of moving retinal images, an appropriate cardboard frame being so placed that the visual field was stationary during rotation. In the normal animal when the action of moving retinal images is excluded the rotatory nystagmus is diminished and post-rotatory increased. Applied to the unilaterally labyrinthectomized animal the conditions are somewhat different; namely, when the direction of rotation is toward the intact side, the rotatory reaction is strong, but not as strong as when the visual field is moving, but the post-rotatory is weak or absent. On the other hand, during rotation toward the operated side rotatory compensation is lacking, or slight, but post-rotatory remarkably accentuated. Here again then we have the algebraic summing of like and unlike effects mediated by the eyes and the labyrinths coupled with the algebraic summing of the operative and rotational effects.

Some observations were made of the reactions to rotation by hand, moving the table a variable amount, a few centimeters to a quarter turn, and so forth. These were very clear and instructive. Nystagmus always occurs upon movement toward the good side of the animal, but seldom during movement toward the injured side. Post-rotatory nystagmus occurs when the movement has been toward the injured side, but not following rotation toward the good side. A considerable number of observations were also made on the eye movements during and following the tilting of normal and unilaterally labyrinthectomized rabbits around the transverse axis, downward and upward, and around the long axis, sideways, the head being held in a plane horizontal to the board. The results on the normal animals need not detain us, the eye-balls deviating, with nystagmus in the opposite direction, in such a manner as to counteract the effect on the field of view of the altered position of the body in space. When unilaterally labyrinthectomized rabbits are tilted, the eye on the sound side behaves like that of the normal animal, but the eye on the operated side seems unable to compensate for changes in the position of the body in space and moves passively in the direction of tilting. Nystagmus accompanies the deviation in practically all cases. In the eye on the operated side this was frequently of a quivering or tremulous nature.

The conclusion drawn from these observations is, that while each labyrinth has a binocular influence there is evidence of differential reduction in tone when one has been removed, as exhibited by the difference in

reaction according as to whether the direction of rotation is toward the intact or operated side. Each labyrinth controls the responses of both eyes to rotational movements in both directions, but in unequal amounts, the control being greater for the eye on the same side. This state of affairs holds for larval and adult Amblystoma, for the frog and for the rabbit.

SUMMARY

Rapid and slow rotation of unilaterally labyrinthectomized frogs and rabbits produces in the majority of cases when the direction of rotation is toward the intact side rotatory, but no, or but slight, post-rotatory nystagmus; and when the direction of rotation is toward the injured side post-rotatory, but no, or but slight, rotatory nystagmus. When a blinder is employed so that the action of moving retinal images is excluded the rotatory and post-rotatory movements are diminished or increased according to the direction of rotation; namely, when rotation is toward the normal side the rotatory reaction is considerable, though not as much so as when the animal sees the moving visual field, while the post-rotatory movements are diminished. When the direction of rotation is toward the operated side the compensating movements are slight or entirely lacking, but the post-rotatory movements are very extensive and numerous.

When unilaterally labyrinthectomized rabbits are tilted forward, backward and sideways, the eye on the operated side, instead of deviating in the normal manner as the other eye does, that is in a direction opposite to that of the tilting with periodic nystagmic jerks in the other direction, rolls passively in the same direction as the tilting.

Each labyrinth functions for both sides but to a greater extent for its own.

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THE REGULATION OF CIRCULATION

V. The Effect of Anemia and Hemorrhage on the Cardiac Output of Dogs[1]

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Received for publication October 14, 1926

Present knowledge of the factors controlling the output of the heart is relatively meagre. Boothby (1), in 1915, suggested that the hydrogen ion concentration of the blood is an important controlling factor, and Douglas and Haldane (2) have presented evidence for this view. Blalock, Harrison and Wilson (3) found acidosis and increased cardiac output in experimental respiratory obstruction. They also (4) observed an increased output of the heart after injecting acid, and a diminished output after injecting alkali.

Leegard (5) found an increased circulatory minute volume in rabbits with experimental pneumonia. We have obtained (6) similar results in dogs, although no significant alterations in the hydrogen ion concentration of the blood were encountered. These results indicated that the acid-base balance could not be the sole factor in the regulation of circulation.

The dogs with pneumonia had increased metabolic rates and anoxemia. Many developed anemia. These three conditions have been studied separately. Changes in metabolic rate produced by feeding thyroid and by thyroidectomy were found to be associated with significant changes in the output of the heart (7). Similar results have been reported by others (8), (9), (10).

The present problem deals with the effects of deficient oxygenation of the blood on the cardiac output. Oxygen deficiency has been produced by the removal of blood and by anoxic anoxemia. Anemia as used in this paper indicates the repeated loss of small quantities of blood over a long

[1] On order to coördinate our previous reports with our major problem this general title has been adopted. Our previous communications, (a) The Effects of Partial Respiratory Obstruction on Circulation and Respiration of Morphinized Dogs, (b) The Effects of Changes in Hydrogen Ion Concentration on the Blood Flow of Morphinized Dogs, (c) Cardiac Output in Pneumonia in the Dog, and (d) The Effects of Thyroidectomy and Feeding Thyroid Substance on the Cardiac Output of Dogs, are to be considered as papers I, II, III and IV of this series.

period of time, whereas hemorrhage implies the rapid removal of larger quantities of blood. The results are reported in this and the succeeding paper. The possible clinical application of the findings will be discussed elsewhere.

Krause (11) and Plesch (12) observed increased cardiac output in severe anemia. Harrop (13) found that the percentage saturation of the venous blood with oxygen was normal in three patients with severe anemia and the coefficient of utilization of oxygen (arterio-venous difference) was greatly decreased. His observations indicated that the blood flow through the arm was probably increased. Similar results were reported by Eppinger, von Papp and Schwarz (14). Lundsgaard (15) found normal coefficients of utilization of oxygen in blood from the arms of anemic patients and believed that the cardiac output does not change as long as the hemoglobin is above 30 per cent. He stated, "The resting anemic organism does not increase its circulation until all the reserve oxygen is used."

Liljestrand and Stenstrom (16) reported increased cardiac output in patients with anemia of varying degree. Dautrebande (17) observed no change in cardiac output as long as the hemoglobin was above 50 per cent. Between 40 and 50 per cent, the cardiac output was moderately increased, and below 40 per cent a very marked increase was noted. Therefore, most of the workers in this field are agreed that anemia increases cardiac output, but considerable uncertainty exists as to the degree necessary to excite this effect. Our interest has centered particularly in the mechanism of this phenomenon, a phase which has received less attention.

METHOD. Dogs were given 6 to 10 mgm. of morphine sulphate per kilo of body weight. One and one-half to two hours later samples of blood were drawn from the right and left ventricles. The usual precautions against contamination with air were followed. The oxygen consumption was then determined with the Benedict spirometer, to which the animal was connected by a specially constructed rubber mask. Check analyses of the arterial and venous oxygen contents were made, the Van Slyke-Neill (18) manometric apparatus being used.

The hemoglobin was not estimated directly but was calculated from the oxygen content of the arterial blood which was assumed to be 95 per cent saturated. Twenty volumes per cent oxygen capacity was assumed to represent 100 per cent hemoglobin. This method of determining hemoglobin is not absolutely accurate, but is adequate for our purpose.

The cardiac output was calculated from the Fick formula:

$$\frac{\text{cc. of oxygen consumed per minute}}{\text{cc. of oxygen taken up by one cc. of blood in passing through the lungs}} \text{ equals cc. of blood passing through the lungs per minute}$$

In order to be certain that the results obtained were not dependent on morphine, one trained unnarcotized dog was used in twenty experiments. Observations were carried out on three different series of animals and the following general problems were investigated.

a. The effect of repeated loss of relatively small amounts of blood (group I). After one or more control determinations the animal was bled 50 to 150 cc. once or twice a week for a period of six to ten weeks. During this time frequent determinations of cardiac output were made. Bleeding was then stopped and determinations made at intervals for three or four months.

b. The effect of rapid loss of blood without the administration of fluid (group II). After one control determination blood amounting to 1 per cent of the body weight was withdrawn. The procedure was repeated at two-hour intervals until five determinations of cardiac output and four bleedings had been carried out. Thus about one-third to one-half of the calculated blood volume was removed in the course of the experiment.

c. The effect of rapid loss of blood and replacement with saline solution (group III). The procedure was carried out in the same way as that just mentioned except that at the end of each bleeding the blood was replaced by an equal quantity of 0.9 sodium chloride solution administered intravenously.

RESULTS. A. *The effects of loss of 50 to 100 cc. of blood at three- to five-day intervals.* Four animals were studied and fifty-eight determinations of cardiac output were made. The results are shown in figure 1.

The general condition of the animals was not altered to any striking degree. At the time of severest anemia some weakness and apathy were noted. One animal was accidentally killed by the production of hemopericardium and another died of intercurrent disease.

The pulse rate of the trained dog increased as the anemia became more severe. In the morphinized animals the changes in pulse rate were less marked.

Constant variations did not occur in the oxygen consumption which, however, was usually somewhat greater during the period of grave anemia. This was much more striking in the trained dog than in those which received morphine. The oxygen consumption of the unnarcotized dog was almost 100 per cent higher during the period of recovery from severe anemia than during the control observations.

The hemoglobin diminished rapidly. The lowest hemoglobin was 25 per cent. Ordinarily bleeding was stopped after a level of 30 to 40 per cent had been reached, as it was considered desirable to save the animals and study them during the recovery period.

The arterial and venous oxygen contents decreased, the former being diminished in greater degree. The utilization of oxygen was consequently diminished after repeated hemorrhages. The percentage saturation of the

venous blood with oxygen was decreased. The lowest venous saturation was 26 per cent. When anemia was severe the venous saturation was usually between 30 and 45 per cent, whereas the venous blood was 50 to 70 per cent saturated in the control determinations. The percentage of hemoglobin was reduced to a much greater degree than was the percentage

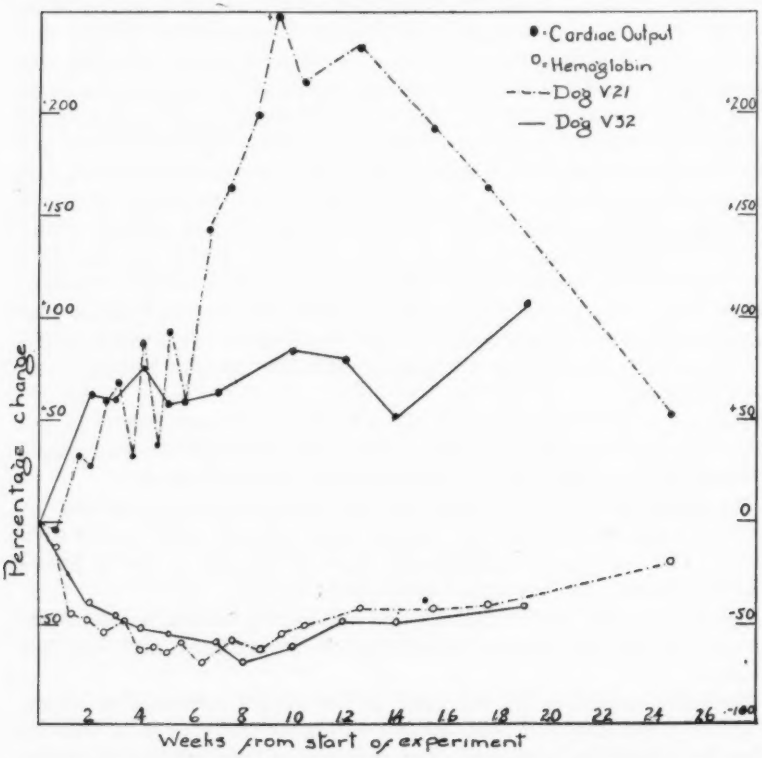

Fig. 1. The results are plotted for the two dogs which showed maximum (V_{21}) and minimum (V_{32}) changes in cardiac output following repeated small bi-weekly bleedings. The most striking feature is that the greatest values for cardiac output occurred during the recovery period—i.e., after bleeding had been stopped.

of saturation of the venous blood. This finding indicates the necessity for a relatively high oxygen pressure in the capillaries and is discussed later. The minute cardiac output increased in every animal, but the degree of increase was variable. The maximum changes in minute cardiac output in the four animals studied were plus 34 per cent, plus 73 per cent, plus 108 per cent, and plus 270 per cent respectively, the greatest increase

occurring in the trained dog. The output per beat also was greater during anemia in all of the animals.

The greatest cardiac outputs were observed during the period of recovery and not at the time when the anemia was most severe. In the two animals which survived, the minute output reached its maximum one and three months respectively after bleeding had been discontinued. The output then gradually decreased, but was still considerably above the control level after a recovery period of four months. These findings are shown in figure 1.

B. *The effects of rapid loss of blood without replacement of fluid.* As described above, these animals were bled amounts corresponding to 1 per cent of the body weight for three or four successive bleedings at two-to three-hour intervals, the cardiac output being determined immediately before each bleeding and two hours after the last bleeding. In two animals subsequent observations were made for several weeks after the immediate experiment.

Twenty determinations of cardiac output were made on three dogs. During the immediate experiments the general condition of the animals was not changed by the first two bleedings, but after the third and fourth bleedings (i.e., after blood amounting to 3 or 4 per cent of the body weight had been removed) some dyspnea and considerable weakness were noted. At this stage the dogs appeared to be in a state of shock.

The pulse rate increased after each removal of blood. No constant variations in oxygen consumption were found. A decrease of 5 to 15 per cent in hemoglobin occurred. The arterial and venous oxygen contents were slightly diminished after the first three bleedings. After blood had been withdrawn a fourth time the venous oxygen was markedly decreased and the coefficient of utilization which had hitherto undergone no significant change was much increased. The cardiac output remained practically constant in two animals after the first three bleedings, but after the fourth bleeding decreased markedly, the decrease in cardiac output corresponding to the appearance of signs of shock. One animal had an initial rise in cardiac output, followed by a fall as the bleeding was continued.

Two dogs were saved and were later studied at intervals. In both of the animals subsequent observations showed that the percentage of hemoglobin was less than at the end of the original experiment. This indicated that the blood volume had increased. The general appearance was entirely similar to that of the animals with the more chronic anemia, the cardiac output was much increased, and remained elevated during the period of recovery from the anemia. As would be expected this period was shorter than in the animals which had undergone more bleeding.

C. *The effects of rapid loss of blood and replacement of fluid.* The observations were carried out in the same manner as those just mentioned, except

that the blood withdrawn was immediately replaced by an equal volume of 0.9 per cent sodium chloride solution, administered intravenously. Sixteen determinations of cardiac output were made on three dogs.

Marked changes in the general condition of these animals were not noted during the immediate experiment. After the fourth bleedings the animals

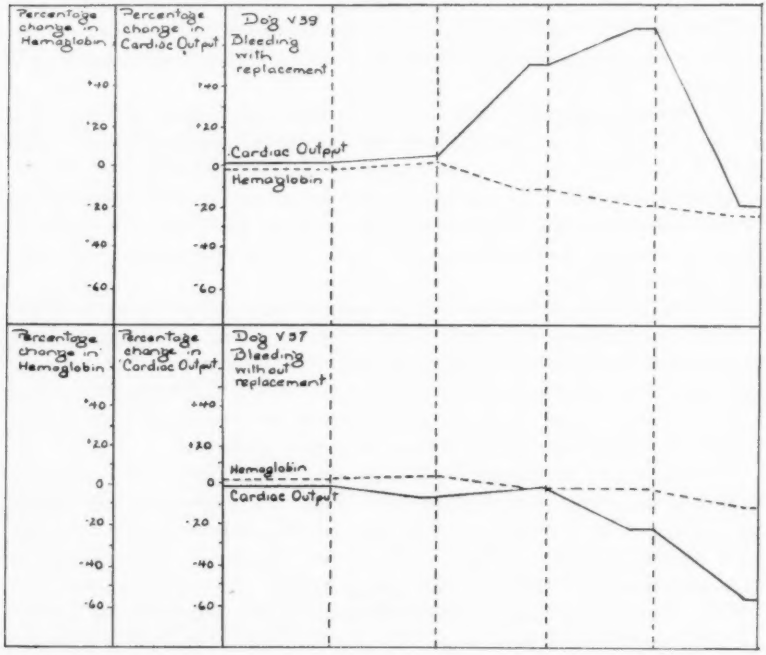

Fig. 2. Two immediate experiments are plotted. In the experiment shown below the blood withdrawn was not replaced by saline solution. The greatest decrease in hemoglobin was 8 per cent. The cardiac output remained practically constant for two bleedings, but after the third and fourth bleeding decreased to 20 per cent and 45 per cent of the original value.

In the experiment shown above the blood withdrawn was replaced by saline solution. The maximum decrease in hemoglobin was 25 per cent. The cardiac output was increased to plus 55 per cent and plus 70 per cent above the control value after the second and third bleedings respectively. After the fourth bleeding the cardiac output was minus 15 per cent. These findings emphasize the importance of hemoglobin concentration and hence capillary oxygen pressure (see text) in the regulation of circulation.

appeared to be in a state of slight shock, but this was less striking than in the dogs which were bled and given no fluid. The pulse rate increased. The oxygen consumption was definitely elevated. The cause of this is not clear unless the increased metabolic rate is to be attributed to the increased

activity of the heart. The hemoglobin decreased during the immediate experiment. The degree of decrease varied in the different animals. The arterial oxygen content decreased 2 to 7 volumes per cent. The venous

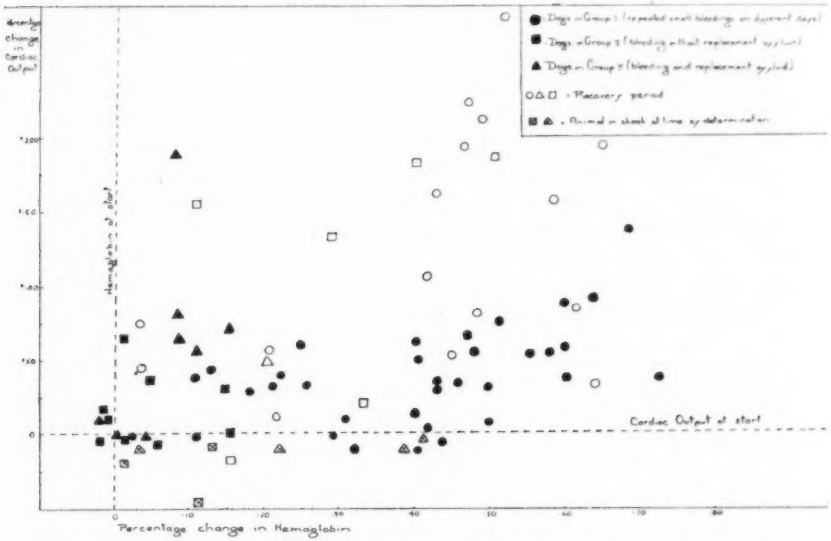

Fig. 3. This chart represents all the determinations of cardiac output made in three groups of animals. The following features are noteworthy:

a. Nearly all the symbols except those containing an x—denoting shock—are above the control line, indicating an increased cardiac output in anemia.

b. The symbols toward the right side of the chart—severer grades of anemia—are, in general, somewhat higher than those at the left.

c. The black triangles (denoting experiments in which the blood withdrawn was replaced by saline) are considerably higher and somewhat farther to the right than the black squares (denoting experiments in which the fluid lost was not replaced). Thus the degree of anemia was greater and the cardiac output was higher in the "replacement" experiments.

d. The black triangles (acute hemorrhage and replacement) are higher than the black circles (chronic bleedings) of the same degree of anemia. Thus time appears to be an important element in determining the degree of cardiac response to a given hemoglobin level. The more rapidly the anemia is produced, provided shock is avoided, the greater the increase in cardiac output.

e. The hollow symbols (denoting recovery periods in the respective groups) are definitely higher than the black symbols. The cardiac output is greatest, therefore, when the animal is recovering from the anemia.

oxygen content changed to smaller degree in most instances and the utilization was usually diminished.

After the first bleeding no significant change in cardiac output was noted, but after the second bleeding a marked increase occurred in every animal.

Following subsequent bleedings the cardiac output remained elevated or decreased according to the degree of shock. The important point is that a significant rise in cardiac output occurred at some period in all experiments in which the blood volume was maintained. That this finding was in contrast to the results obtained when the blood was diminished and the hemoglobin was less changed is shown in figure 2.

The greatest cardiac outputs observed in the experiments in which the fluid was not replaced were plus 63 per cent, plus 15 per cent and minus 3 per cent of the original control figures for these animals. The greatest cardiac outputs found in the experiments in which the fluid was replaced were plus 181 per cent, plus 70 per cent and plus 52 per cent respectively. In the first group of animals the maximum changes in hemoglobin occurring in the immediate experiments were minus 13 per cent, minus 11 per cent and minus 13 per cent respectively. In the second group the maximum hemoglobin changes during the immediate experiment were minus 24 per cent, minus 28 per cent, and minus 8 per cent. (In the latter group the dog which had the smallest change in hemoglobin had the greatest increase in cardiac output. The cause of this is not clear.)

The results found in the three groups of animals are summarized in figure 3. A rather loose correlation is seen to exist between the degree of anemia and the degree of increase in cardiac output. There are three exceptions to this general rule: 1. During the period of recovery the hemoglobin is found to approach the normal level sooner than the cardiac output. 2. During the state of shock which supervened in some of the immediate experiments, the cardiac output decreased despite a diminution in hemoglobin. 3. Anemia produced rapidly causes a relatively greater increase in cardiac output than anemia produced more slowly (black circles—fig. 3).

Discussion. The results of these experiments are in accord with the findings of most other observers, in so far as they indicate that the cardiac output is increased in severe anemia. The essential points to be considered are: 1, the mechanism whereby this compensatory adjustment is brought about, and 2, the degree of anemia necessary to produce a change in the cardiac output. In regard to the first point it is noteworthy that a decreased concentration of hemoglobin was followed by an increased cardiac output, whereas diminution of the total amount of hemoglobin was not necessarily followed by an increase in the cardiac output.

That loss of blood in itself does not materially change the cardiac output is shown by those experiments in which the animals were bled and the blood volume was not restored.

The increased cardiac output was usually associated with significant diminution of the hemoglobin content of the blood and it seems likely that the latter was the cause of the former. Although hemoglobin functions

as a buffer as well as a carrier of oxygen, we believe that only the latter function is important in this connection (see the following paper in the present issue of THIS JOURNAL).

The fact that diminution in the oxygen carrying power of the blood was followed by increase in cardiac output naturally raised the question as to whether the oxygen tension of the blood might not be an important factor in the control of circulation. Unfortunately, the oxygen tension cannot be determined from the data available, as the degree of dissociation of hemoglobin is partially dependent on the carbon dioxide tension (19) which cannot be calculated because determinations of pH and carbon dioxide content were not made. The percentage saturation of the venous blood with oxygen was diminished in all experiments except those in the later stages of the recovery period when the venous saturation was normal. Although the exact values for the oxygen tension are not available, it is reasonably certain that the pressure of oxygen in the venous blood was diminished.

Any regulatory effect ascribable to changes in the oxygen tension of the blood must depend on alterations of the pressure of this gas in the capillaries. Since the capillary oxygen tension is dependent on that of the arterial blood as well as that of the venous blood, variations in the latter alone cannot cause changes of similar magnitude in the capillaries. Lundsgaard and Van Slyke (20) have designated the mean capillary oxygen unsaturation as $\frac{A-V}{2}$ where A represents the arterial unsaturation and V the venous unsaturation. For the sake of convenience we have used a similar scheme and have designated "mean capillary oxygen pressure" as capillary pO_2 which equals $\frac{\text{Art } pO_2 \text{ plus Ven. } pO_2}{2}$. (It must be borne in mind that this is purely a theoretical representation of a possibility. Since the blood flows more slowly through the venous loop than through the arterial loop of the capillaries it is likely that Art. pO_2 minus Cap. pO_2 is greater than Cap. pO_2 minus Ven. pO_2. The above formulation is convenient, however, and holds true if "mean" be used to designate the average of the highest and lowest capillary oxygen pressures and not the average of the sum of all the pressure levels.) A change of 20 mm. in Ven. pO_2 would of course cause a change of 10 mm. in Cap. pO_2.

The oxygen capacity of the blood was not determined in our experiments. It is likely that the variations in arterial oxygen saturation were not great, but as relatively small changes in this factor produce relatively great changes in oxygen pressure—the dissociation curve of oxy-hemoglobin being almost horizontal at 95 per cent saturation—constancy of the arterial oxygen pressure cannot be assumed.

Despite the lack of complete data it is justifiable to believe that the capillary oxygen pressure was at least slightly diminished and it is possible

that this was the cause of the observed increase in cardiac output. There is one important objection to this view. In all of the dogs the cardiac output was greater during the recovery period than during the period of severest anemia (fig. 3). However, in any series of physiological observations the greatest response does not necessarily imply the greatest stimulus, as the ability to respond to a given stimulus may vary according to the degree of functional efficiency of the organ in question. It is well known that loss of a large amount of blood results in circulatory shock, and that long standing anemia produces anatomical changes in the heart muscle. We believe that the dogs had a greater cardiac output during the period of "recovery" because their hearts were then more efficient. It is probable that the functional efficiency of the heart was impaired during the period of gravest anemia and that the organ was unable to respond normally to the stimulus caused by the loss of blood. Consequently it is not felt that the fact that the greatest cardiac outputs were encountered during the "recovery" period is conclusive evidence against the view that capillary oxygen pressure is an important factor in the regulation of cardiac output.

The individual dogs reacted to comparable degrees of anemia with considerable differences in the degree of increase in cardiac output. This was to be expected, as physiological responses to the same stimulus are usually similar qualitatively, but dissimilar quantitatively in different individuals.

In several of the animals an increase in oxygen consumption of considerable degree occurred during the course of the observations. This was often greatest during the recovery period. The cause of this is not clear.

Another point of interest is the relationship between the degree of anemia and the increase in cardiac output. All observers are agreed that anemia may lead to an elevation of the cardiac output, but there is no uniformity of opinion as to what degree of anemia is necessary in order to bring this about. As mentioned above, Lundsgaard (15) claimed that no increase in cardiac output occurred until the hemoglobin approached 30 per cent, while Dautrebande (17) believed 50 per cent of hemoglobin to be the critical level. In observations extending over a period of two years and including several hundred determinations of the cardiac output of supposedly normal dogs, we have frequently encountered animals with a moderate degree of anemia (hemoglobin of 60 to 70 per cent) and with cardiac outputs which fell within the limits of normal (100 to 200 cc. per kgm. per minute). E. K. Marshall has reported similar observations (personal communication). This finding suggests that anemia must be of severe grade, below 60 per cent hemoglobin, before leading to any change in cardiac output. However in figure 3 there are depicted numerous observations which point to an elevation of cardiac output with degrees of anemia con-

siderably milder than this, and this supports the findings of Liljestrand and Stenstrom (16).

The two points of view are not entirely irreconcilable because the time element seems to play an important rôle. When the circulation was not handicapped by a diminished blood volume or by shock from excessive bleeding, the cardiac output was increased in the immediate experiments even with relatively slight degrees of anemia (fig. 3, black triangles). When the bleeding extended over a longer period of time, relatively greater degrees of anemia were necessary before the cardiac output increased (fig. 3, black circles). In dogs which are anemic but otherwise apparently normal, it is altogether likely that the anemia has required considerable time to develop and adjustment has occurred without any change in the cardiac output. It is believed that these conceptions as to the importance of the time element explain the conflicting views mentioned above.

The results found in this work cannot be attributed to morphine, as the greatest changes in cardiac output occurred in the trained unnarcotized animal.

SUMMARY

The effects of anemia and hemorrhage on the cardiac output of intact dogs have been studied by the Fick method. Diminution of the hemoglobin content of the blood is associated with an increased cardiac output. Progressive hemorrhage leading to decreased blood volume causes no marked change in cardiac output until the animal approaches a state of shock when the cardiac output rapidly decreases. When blood volume is maintained by saline infusion, progressive hemorrhage is associated with an increased cardiac output, which diminishes as shock sets in.

These effects occur in animals narcotized with morphine and in trained unnarcotized animals. The time element is of considerable importance. The more rapidly a given degree of anemia is produced the more is the output of the heart increased.

It is suggested that capillary oxygen pressure may be an important factor in the regulation of the output of the heart.

We take pleasure in expressing our appreciation to Dr. Glenn E. Cullen for helpful suggestions in regard to this work.

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THE REGULATION OF CIRCULATION

VI. The Effects of Severe Anoxemia of Short Duration on the Cardiac Output of Morphinized Dogs and Trained Unnarcotized Dogs

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Received for publication October 14, 1926

In the preceding paper a study of the effects of anemia and hemorrhage on the output of the heart has been reported. Reduction of the hemoglobin concentration was found to be followed by an increase in cardiac output, the degree of increase being relative to the severity of the anemia, and to the rapidity with which it was produced. These findings suggested that the oxygen pressure in the capillaries might be an important factor in the regulation of the cardiac output. Accordingly studies have been undertaken with the object of determining whether deficient oxygenation of the blood, due to anoxic rather than anemic anoxemia, is associated with changes in the circulatory minute volume.

The problem of the cardiac response to deficiencies in the transport of oxygen is an interesting one and previous observations along this line have not been lacking.

Schneider and Truesdell (1) observed an increased pulse rate and increased pulse pressure but no change in the blood flow through the hand, in normal individuals in whom sudden and extreme anoxemia was produced by rebreathing. The same authors (2) reported an increased pulse rate and pulse pressure and decreased blood flow through the hand and peripheral capillaries when gradual severe anoxemia was produced by rebreathing and when constant moderate anoxemia was present. As a result of their investigations Schneider and Truesdell stated: "It is concluded that the circulatory changes in anoxemia do not serve as a means of compensating for a lack of oxygen, but that they may be interpreted as signals of distress." Doi (3), using the Fick method, found no change in the cardiac output of urethanized cats when the percentage of oxygen in the inspired air was as low as 13.31 and the arterial blood 81 per cent saturated with oxygen.

Dreyer (4) made cardiometric studies on decerebrate cats and found an increased cardiac output after one to three hours of anoxemia, the arterial

blood being 50 to 80 per cent unsaturated. As a result of hemodynamic studies on dogs narcotized with morphine and chloretone, Sands and DeGraff (5) stated: "Increased systolic discharge, an increased rate of the heart, and a reduced peripheral resistence all combine during anoxemia to increase the minute flow of blood through the body." In studying progressive anoxemia these authors found that eventually a "circulatory crisis" occurred, after which the cardiac output was diminished.

These discrepancies are to be attributed to the fact that the methods used were indirect and the animals had been given narcotics and subjected to various operative procedures. Blalock (6) has shown that ether has a very marked effect on the cardiac output, and Marshall (7) found that urethane alters the response of the heart to changes in rate. In view of their findings, it is logical to believe that any observations on the cardiac output of anesthetized or narcotized animals cannot be regarded as entirely applicable to normals, unless checked by similar observations on unanesthetized trained animals. Marshall has demonstrated that such studies are feasible.

As the relationship of anoxemia to the minute output of the heart is a complicated problem, it has seemed advisable to carry out the study from several different points of view. In this paper the results of severe anoxemia of short duration are reported. In subsequent publications the effects of milder degrees of anoxemia of long and short duration will be presented, and the possible clinical application of the findings will be discussed.

Although there is no sharp dividing line between severe and mild anoxemia, in these studies we have arbitrarily classified an arterial saturation of less than 70 per cent as "severe" and have considered saturations of 70 to 90 per cent as "mild."

Method. Eight experiments including twenty-four determinations of cardiac output were carried out on morphinized dogs. These animals were given the drug—6 to 10 mgm. per kilo of body weight—approximately one and one-half hours before the start of the observations. Five experiments including thirteen determinations of cardiac output were carried out on trained unnarcotized dogs.

Initial control determinations were made in every instance, the method used being that described in the preceding paper. It was found advisable to make the blood oxygen determinations as soon as the blood was drawn, for some passing of oxygen from the oil into the blood occurs if the samples are allowed to stand.

As soon as the control determination of cardiac output had been completed the Benedict spirometer was filled with room air and the animal attached. The soda lime filter was left in situ and consequently the carbon dioxide was absorbed and the animal breathed an oxygen-nitrogen

mixture in which the percentage of the former gas was steadily decreasing. The duration of rebreathing was varied in different experiments according to the size of the animal and the degree of anoxemia desired. After seven to forty-five minutes of rebreathing the right and left ventricles were punctured simultaneously by two different observers. The blood samples were immediately analyzed for oxygen content and, in some instances, for carbon dioxide content.

The oxygen capacity was determined in most of the experiments, but in a few the control arterial blood was assumed to be 95 per cent saturated and the oxygen capacity calculated.

The oxygen consumption during rebreathing was calculated from the spirometer tracing, just as in the control periods. The minute ventilation was calculated from the same tracing, the rate of respirations being multiplied by the average depth of the respirations. The values obtained by this method are only approximately correct. No calculations of the minute ventilation were made for the morphinized dogs.

The pulse rate was counted immediately before blood samples were drawn.

Hydrogen ion determinations were made in several experiments, the method of Hastings and Sendroy (8) being used. In some experiments only one control determination and one determination during anoxemia were made. In other experiments a second control was made thirty to sixty minutes after the cessation of anoxemia. In several experiments anoxemia was again induced.

Results. The dogs remained quiet during most of the observations. Two experiments were discarded because the animals struggled when blood samples were being drawn.

As morphine causes slowing of the heart and affects the breathing of dogs the changes in pulse rate and in ventilation are not considered entirely reliable in the dogs to which the drug had been given. The heart rate of the trained animals was slightly augmented during anoxemia—the average increase being about ten beats per minute. The changes in respiratory rate were variable and usually not striking. During anoxemia the respiratory depth was usually somewhat greater and the minute ventilation was increased in five of the six observations on trained dogs. As the changes in pH were slight and usually in the direction of increased alkalinity, these observations suggest that anoxemia may be of considerable importance in the regulation of the respiration, and are more nearly in accord with the views of Gesell as expressed in his recent review (9) than with other conceptions of respiratory control.

The changes in oxygen consumption are of some interest. Variations of less than 15 per cent are not to be ascribed to anoxemia as animals may vary to at least this degree in the course of one or two hours on the

table. In one experiment a rise of 40 per cent occurred in the oxygen consumption, the arterial blood being 50 per cent saturated. For this no explanation is offered. In general no significant change was noted in oxygen consumption when the arterial saturation was more than 50 per cent. When the arterial saturation was less than 40 per cent a definite diminution in oxygen consumption was found, the degree of diminution being roughly proportional to the severity of anoxemia.

In the control observations the arterial oxygen contents were between 12 and 20 volumes per cent and the arterial blood was 90 to 100 per cent saturated. The venous oxygen contents in the control observations were between 7 and 15 volumes per cent. The utilization of oxygen was not lower than 4.4 volumes per cent in any of the initial control determinations, and was between 5 and 7 volumes per cent in most instances.

The degree of diminution of the arterial oxygen content during the anoxemic period was variable. The lowest value found was 0.63 volume per cent. This corresponded to an arterial saturation of only 2.9 per cent. In the same experiment the venous oxygen content was 0.02 volume per cent, which may be considered as zero since the mean variation of check determinations of blood oxygen content is, in our hands, about 0.1 volume per cent.

During anoxemia the utilization (A-V) was unchanged in one instance and was less than the control value in fifteen instances. The highest, lowest and average values found for the utilization during severe anoxemia were 5.3, 0.6 and 2.4 volumes per cent respectively.

The oxygen capacity was assumed to be constant in five experiments, but in the others was determined each time blood samples were drawn. The changes found in oxygen capacity were usually only slightly greater than the error of the method of analysis. (In one experiment a decrease of 3.5 volumes per cent was found. This may have been due to error as single determinations of oxygen capacity were considered sufficiently reliable, whereas duplicate analyses of arterial and venous oxygen content were always made.)

The carbon dioxide content of the blood was determined in eight experiments. During anoxemia an increase was found in two instances and a diminution in six instances. Although morphine may affect the CO_2 combining power of the blood, the changes in CO_2 content observed in these experiments were similar in the morphinized and in the trained animals and therefore cannot be attributed to the drug. In five of the experiments carried out on morphinized dogs hydrogen ion determinations were made. The control values fell within the limits (7.15 to 7.35) found as normal by Harrison, Wilson and Blalock (10) for dogs to which the drug had been given. During anoxemia the pH increased in five instances and decreased in one. The changes were never very striking, the maximum variation being pH 0.08.

These changes in CO_2 and pH during anoxemia are to be interpreted as indicating a condition of uncompensated acid deficit and correspond to Van Slyke's area "3" (11). Such changes are to be attributed to over ventilation. Koehler, Brunquist and Loevenhart (12) reported a similar slight alkalosis in dogs during the initial stages of anoxemia of long duration. Their animals later developed acidosis, but only after having been anoxemic for a period considerably longer than the duration of the experiments reported in this paper.

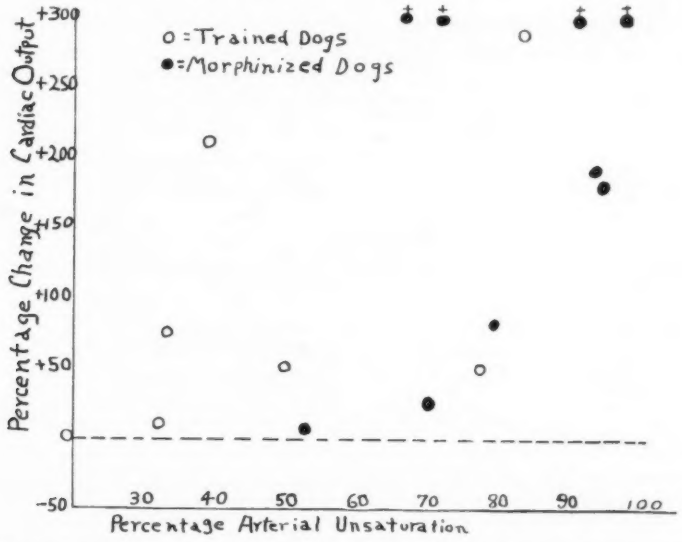

Fig. 1. The percentage change in cardiac output is plotted against the percentage of arterial unsaturation. In every instance the cardiac output during anoxemia is above the control—zero—line. In general the output is greater during severe than during mild anoxemia. No striking difference can be observed between the normal and the morphinized dogs.

In the control periods the minute cardiac output fell within the limits which have previously been found as normal for trained and morphinized dogs—100 to 210 cc. per kgm. per minute (7), (10). During anoxemia the output of the heart increased in every instance, but the degree of change was very variable in the different dogs as well as in different observations on the same animal. The maximum and minimum changes found in cardiac output were +539 per cent and +5 per cent respectively.

The degree of increase was, in general, more or less in proportion to the degree of anoxemia. This is shown in figure 1 in which the percentage

change in cardiac output is plotted against the degree of anoxemia, and in figure 2, in which the output per minute per kilogram of body weight is plotted against the degree of anoxemia.

The increase in cardiac output was chiefly due to an augmented stroke volume as the changes in pulse rate were relatively slight.

The cardiac output one to two hours after the end of the anoxemic period was less in every instance than that during rebreathing. In the seven experiments in which second control determinations were made the

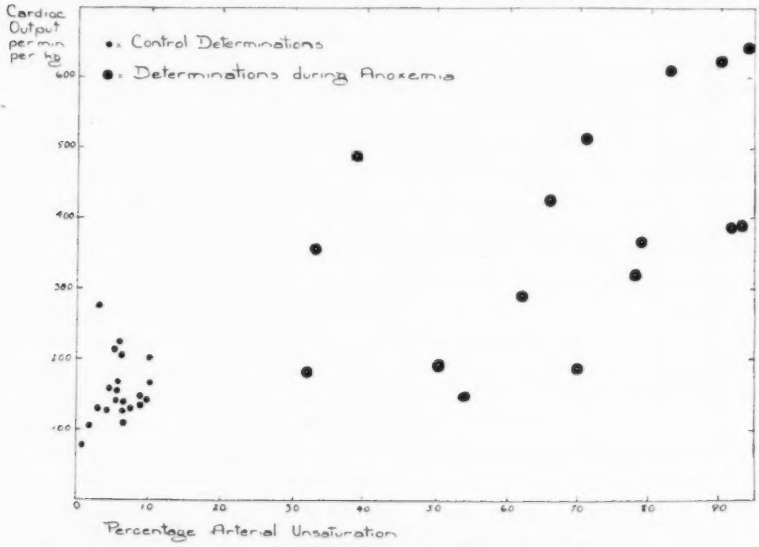

Fig. 2. The small circles denote control determinations and in these—with one exception—the cardiac output was between 100 and 220 cc. per kilo per minute and the arterial blood 90 to 100 per cent saturated with oxygen. The large circles denote determinations made during varying degrees of anoxemia. Most of the cardiac outputs are above the upper limit for the control determinations. The degree of increase in cardiac output corresponds in a general way with the degree of anoxemia.

cardiac output was twice found to be greater and twice found to be less than that during the initial control period, and in the other three instances no change was found. The conclusion is drawn that severe anoxemia of short duration exerts no constant after effect on the function of the heart.

Discussion. These experiments constitute the first studies of the effect of anoxemia on the total minute blood flow of unanesthetized, unnarcotized animals. In view of the uniform results as contrasted to the conflicting findings of those who studied the blood flow through parts of the body only, or who have used anesthetized animals which had been

subjected to various surgical procedures, it appears justifiable to conclude that the output of the normal dog's heart is always increased when the arterial blood is less than 70 per cent saturated with oxygen.

It has been previously shown (10) that variations in H-ion concentration cause changes in cardiac output. The findings in the present observations can not be ascribed to acidosis, however, because the changes in H-ion concentration were usually in the direction of increased alkalinity, which tends to decrease the total blood flow. Furthermore the variations in pH occurring in the present observations were of smaller magnitude than those which were found to lead to alterations in the cardiac output.

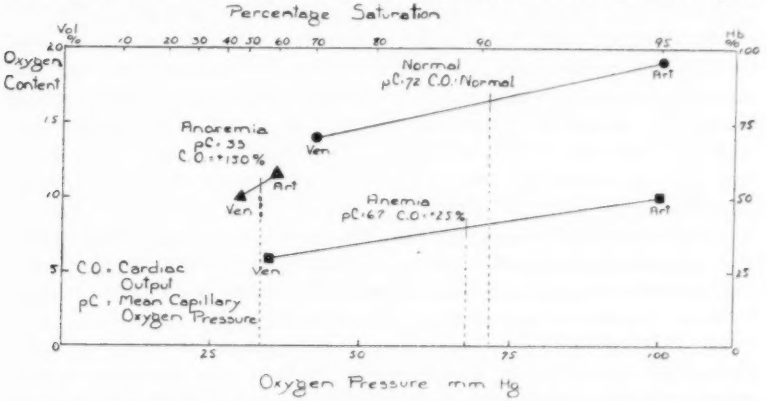

Fig. 3. One hundred per cent hemoglobin is assumed to correspond to 20 volumes per cent oxygen capacity. The oxygen content of the blood is plotted against the oxygen pressure. The percentage saturation of the blood for any given oxygen pressure has been derived from Bohr's dissociation curve—40 mm. CO_2 pressure being assumed—and plotted above. The chart is schematic but represents the condition found in typical experiments. The object of the chart is to contrast the capillary oxygen pressure in anemia (50 per cent hemoglobin) and anoxemia (60 per cent arterial saturation). In anemia of this degree the "mean capillary oxygen pressure" (see text) is slightly decreased and the cardiac output is slightly increased. In anoxemia the changes in both functions are much greater.

The evidence indicates that the changes found in the latter are to be ascribed directly to anoxemia. In the previous paper on the effects of anemia and hemorrhage, the suggestion was made that the capillary oxygen pressure was probably an important factor in the regulation of the circulation. A comparison of the data from the two studies shows that anoxemia caused a very much greater change in cardiac output than did anemia of corresponding degree. This is entirely in accord with the hypothesis concerning the importance of capillary oxygen pressure in circulatory control. In severe anemia the pO_2 in venous blood is decreased

but the arterial oxygen pressure is not altered and consequently the change in mean capillary oxygen pressure is considerably less than in anoxemia in which reduction occurs in both venous and arterial pO_2. These conceptions are shown diagrammatically in figure 3.

These data concerning the qualitative and quantitative effect of anoxemia on cardiac output support the idea that capillary oxygen pressure plays an important rôle in the regulation of circulation, but such an hypothesis can not be regarded as proven until observations concerning the effect of anoxemia of lesser severity and longer duration have been made. Such studies are in progress at the present time.

It is of some interest to consider the mechanism whereby variations in oxygen pressure may conceivably alter the circulatory minute volume. Observations on acidosis (10) suggested that the increased cardiac output was probably dependent, in part at least, on capillary dilatation and diminished peripheral resistance. Krogh (13) found that oxygen lack produced dilatation of the capillaries of the frog's tongue, and it is possible that such an action may play a rôle in increasing the cardiac output in anoxemia.

Another possibility is the existence, in addition to the vasomotor center, of a medullary circulatory center, analogous to the respiratory center, which effects the cardiac output through the nerves to the myocardium. Evidence for such a mechanism is lacking at present.

The third possibility is that changes are brought about by a direct myocardial action of the blood passing through the coronary vessels. Anrep and Bulatao (14) have shown that an increased coronary flow causes an increased cardiac output, while Hilton and Eichholtz (15) demonstrated that anoxemia increases the coronary flow. Present evidence indicates therefore that anoxemia increases cardiac output by a direct myocardial action. The question as to whether in the final analysis this effect is due to oxygen lack *per se* or to the presence of unoxidized acid metabolites remains unanswered.

The maximum increase in cardiac output observed in these experiments has been approximately 300 to 500 per cent, or an increase to fourfold and sixfold respectively. Ordinarily in healthy dogs the coefficient of utilization is 25 to 35 per cent of the total arterial oxygen content. If an animal is able to quintuple his cardiac output and can use all the reserve oxygen in the blood then the greatest possible oxygen intake should be fifteen to twenty times the resting oxygen consumption. Such a conclusion is substantiated by the observations of Hill and Lupton (16) who found that the maximum oxygen consumption of men during running was 3500 to 4000 cc. per minute, which is about fifteen times the resting oxygen consumption. From their findings these investigators calculated a maximum cardiac output during exercise of about 30 liters per minute

for the normal male. As 5 liters per minute is, according to the investigations of Burwell and Robinson (17), approximately the average normal cardiac output of adult men, this represents a maximum increase of 500 per cent. This corresponds well with the maximum cardiac output which we have found for dogs with anoxemia, and indicates that findings in these animals can probably be applied with some accuracy to men.

Great increase in the minute output of the heart, with relatively little change in the pulse rate, necessarily postulates an increased cardiac diastolic volume, and Takeuchi (18) has shown that anoxemia causes such a change in the size of the heart.

In order to consider the application of these results to human physiology it is necessary to assume that anoxemia affects the hearts of man and dog in the same manner. The foregoing discussion has been based on such an assumption.

SUMMARY

Anoxemia has been produced by rebreathing, the CO_2 being absorbed. The effects of arterial oxygen saturations of 3 to 70 per cent on the cardiac output of morphinized and trained dogs have been studied by the Fick method. Anoxemia of severe degree (greater than 30 per cent unsaturation) causes the following changes:

1. Slight increase in pulse rate.
2. Increased minute ventilation.
3. Diminished oxygen consumption (when the arterial saturation is less than 50 per cent).
4. Uncompensated acid deficit (diminished CO_2 content and decreased H-ion concentration).
5. Increase of 5 to 500 per cent in minute cardiac output.

These findings support the hypothesis that capillary oxygen pressure is an important factor in the regulation of the circulatory minute volume.

We desire to express our appreciation to Dr. E. K. Marshall, Jr., who first suggested this problem, and whose encouragment has been a helpful factor in its solution.

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THE BEHAVIOR OF DYES IN THE KIDNEY TUBULE OF NECTURUS

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Received for publication October 14, 1926

The pelvic or functional kidney of Necturus contains on its ventral surface close to the row of pear-shaped glomeruli visible there, nephrostomes which open directly from the abdominal cavity through short canals into proximal convoluted tubules. These nephrostomes, depicted by Chase (1923) and subsequently turned to physiological account by the experiments of Dawson (1925), have made possible the experiments, the results of which are presented here.

Dawson studied the distribution of iron salts in the kidney: *a*, after intraperitoneal injections,—allowing 2 to 24 hours to elapse before anesthetizing the animals, tying off the blood supply to the kidney, removing the kidneys, fixing them in formalin acidified with 2 per cent hydrochloric acid and serially sectioning them; *b*, after intravenous injections,—allowing 1 to 9 hours to elapse before fixing and sectioning the kidney as in *a*. By this method he concluded that iron salts which reach the lumina of the tubules from the body-cavity are absorbed by the epithelium of their proximal and distal convoluted portions. But iron salts reaching the kidney from the blood stream are eliminated by the glomeruli and reabsorbed (indirect evidence) by the epithelium of the convoluted tubules.

The results of experiments presented here also involve intraperitoneal and intravenous injections, but of dyes rather than of iron salts and the observation of the living kidney rather than of fixed material. The dyes injected are chiefly those which have been carefully selected after several years of experimental study and found to be readily excretable and apparently innocuous. The undertaking of these experiments on the kidney of Necturus with dyes was primarily with the intent to discover what happens when they get into the lumen of the tubule by way of the nephrostomes and not by way of the blood stream.

For this purpose Necturi were anesthetized by placing them for an hour or more in a 1 to 2 per cent solution of urethane. The dye was then injected intraperitoneally either before or after dissection to expose the kidney. Sometimes intraperitoneal injections were made without anesthesia and the animal subsequently anesthetized in order to expose the kidney and observe it under the microscope.

Intravascular injections were also made in the same animal following the observation of the results of the intraperitoneal. Thus by consecutive intraperitoneal and intravascular injections of the same dye into the same animal as well as by using the same dye now for intraperitoneal, now for intravascular injections, some definite results were obtained.

All observations were made under a compound microscope with reflected light from an electric lamp and condensing lens. The animal was placed in a paraffined box and enough Ringer's solution containing urethane added to cover the kidney and gills. Solutions of the dyes used were also obtained by using Ringer's solution instead of distilled water. Magnifications of 75, 185 and 740 diameters were obtained as desired.

The following dyes were used for both intraperitoneal and intravascular injections: phenol red, tetrachlorphenol red, dibromphenol red, fluorescein and buffalo fast crimson. In addition to these, but for intraperitoneal injections only, light green SF, buffalo black, trypan blue and toluidine blue were used.

In twenty experiments performed at various times from December 1925 to April 1926, tetrachlorphenol red was injected (1.5 to 2 cc.) intraperitoneally as follows: 1. In 4, the dye was injected into the abdominal cavity of the unanesthetized animal by using a hypodermic needle. After 2 to 5 hours the animal was anesthetized, dissected to expose the kidney, the body-cavity washed free of the remaining dye with Ringer's solution and the kidney observed under the microscope. 2. In 5 experiments, the animal was first anesthetized, placed on its back, injected, and allowed to remain thus from 2 to 4 hours in water containing urethane after which the kidney was exposed and studied. 3. In 11 experiments the animal was anesthetized, the kidney exposed and the dye placed on it with a capillary pipette or enough in the body-cavity to cover it.

In all of the above experiments, dye was observed in the lumina of certain tubules visible on the ventral surface. It remained visible for varying lengths of time before it was washed out along with the flow of urine through the tubule. At no time, as a result of an intraperitoneal injection, was the dye seen to be appreciably absorbed by any of the cells of the tubules.

In 15 of the 20 experiments in which tetrachlorphenol red was injected, the circulation (which is frequently poor in winter Necturi) through the kidney was adequate for an intravascular injection. The results of intravascular injections are also definite and as follows: within 5 to 10 minutes the dye is seen in both cells and lumina of the proximal convoluted portions of the tubule. The intensity of staining varies somewhat depending apparently on the functional state of the kidney. Kidneys in which the circulation is slow show little or no staining of their tubules. Kidneys in

which the circulation is good show, typically, definite staining of the cells of the proximal tubules as indicated in the following protocol:

2:00 p.m. Necturus (male) placed in a 2 per cent solution of urethane.
4:00 p.m. Necturus placed on his back and pinned down. Short incision made through abdominal wall and 1.5 cc. of a 0.6 per cent solution of tetrachlorphenol red injected into the peritoneal cavity.
5:10 p.m. Dye seen in urine from cloaca. Kidney exposed and peritoneal cavity washed free of dye with Ringer's solution.
5:20 p.m. Kidney observed. Circulation excellent. Visible segments of tubules connecting with nephrostomes contain dye in their lumina.
5:25 p.m. Only traces of dye here and there in the lumina of a few tubules. Cells of tubules not visibly stained.
5:45 p.m. Intravenous injection (hepatic vein) of 1.5 cc. of a 0.6 per cent solution tetrachlorphenol red.
6:00 p.m. Circulation good,—cells and lumina of proximal convoluted tubules are of a uniform purple color. Distal convolutions seem to have dye only in the lumina of the tubules.
6:15 p.m. Kidney excised and transected.
6:30 p.m. Cross-section of kidney observed along cut surface. Cells of all the proximal tubules stained purple with dye. The cells of certain tubules normally containing yellow pigment seem less intensely stained than those without pigment,—this difference probably owing to the presence of the pigment.

Six experiments were performed in which phenol red was injected as indicated in the above protocol of tetrachlorphenol red, i.e., intraperitoneally and intravascularly. Four in which dibromphenol red was injected, three with buffalo fast crimson and two with fluorescein. The results from injecting these four dyes intraperitoneally as well as intravascularly are essentially the same as those obtained from similar injections of tetrachlorphenol red. They are not absorbed from the lumina of those tubules permitting the entrance of dye through the nephrostomes as is indicated by the fact that they do not stain the cells of the tubules whose lumina they border on. They visibly stain the cells of the proximal convoluted tubules when they are injected intravascularly.

One or two experiments each were carried out with intraperitoneal injections only of trypan blue, light green SF, buffalo black and some other indifferently excretable dyes. The invariable observed result of intraperitoneal injections of any of the dyes used is that the dye gets into the lumina of certain of the tubules through nephrostomes, but are not absorbed from their lumina in appreciable amounts. This is seen to occur almost immediately when the dye is applied directly to the ventral surface of the kidney even in amounts so small that only two or three nephrostomes are covered by it.

That the dye enters the tubule through a nephrostome and not otherwise is indicated as follows: 1. By its presence in the lumina of tubules

connecting with nephrostomes and by its absence in the lumina of tubules not connected with them. 2. The time between the injection of the dye and its appearance in the lumina of certain tubules is not sufficient to permit absorption from the body-cavity. 3. Only certain of the tubules on the ventral surface contain dye in their lumina as a result of an intraperitoneal injection, whereas all of the proximal tubules contain dye in both cells and lumina as a result of an intravascular injection.

It is to be noted that dyes entering the tubule directly through nephrostomes are in greater concentration than when they are injected intravascularly since a 0.6 per cent solution of all dyes mentioned was used. Dyes entering the lumen of a tubule from the body-cavity are in full 0.6 per cent strength, whereas those injected intravascularly have already been diluted by the blood when they first reach the kidney. Yet with this greater concentration no appreciable absorption from the lumina of the tubules occurs.

None of the dyes thus far mentioned is visibly absorbed from the lumen of the tubule in which it is seen as a result of an intraperitoneal injection. Toluidine blue, however, since it requires special mention, is dealt with separately. This dye stains living tissue cells intensely and distinctively whenever and wherever it comes into contact with them. Its color, when tissue is thus stained by it, varies from marine blue to royal purple. This variation in color is probably caused by changes in pH. It stains certain tissues more intensely per unit time than it does others. It seems to concentrate in or around the nuclei of cells thus giving the appearance of fixed tissue stained with Delafield's hematoxylin. When it is applied directly to the surface of the kidney, almost immediately the nephrostomal tissue is stained intensely by it. The renal tubules stain as readily but not so intensely unless the dye remains on the kidney for a long time. It is absorbed as readily from the lumen of a tubule as from its periphery. This is revealed by observing the kidney after intravascular as well as intraperitoneal injections and also by observing transections of the kidney made after intraperitoneal injections and local applications of the dye to it.

The following protocol gives the main features in the behavior of toluidine blue when it is injected intraperitoneally or intravascularly:

10:30 a.m. Necturus (male) placed in a 2 per cent solution of urethane.
11:30 a.m. One cubic centimeter of 0.6 per cent solution of toluidine blue injected intraperitoneally with a hypodermic needle. Animal kept with gills immersed in a 1.5 per cent solution of urethane.
1:00 p.m. Kidney exposed and dye washed from body-cavity. Nephrostomal discs clearly outlined as seen macroscopically.
1:10 p.m. Kidney observed under microscope. Nephrostomal epithelium stained more intensely and uniformly than peritoneal epithelium. The color of the dye in the cells of the nephrostomal tubule is deep purple. The

cells of the renal tubules are stained light blue as to cytoplasm and deep blue as to nuclei. Observation of the lumina is clouded by the presence of dye in the cells. Intestine and portion of liver stained like tubules.

2:10 p.m. Kidney excised and transected. Transections observed under microscope. Dorsal and ventral tubules stained similarly, but dorsal not stained so intensely as ventral. This difference is owing to relative lack of contact with dye since dorsal tubules have no nephrostomes and are closely applied to the body wall. Tubules in central part of kidney stained very slightly to not at all.

It is perhaps well to restate here that the appearance in the cells of the proximal tubules of Necturus of the dyes, phenol red, etc., is different from that of trypan blue or toluidine blue. The former and readily excretable dyes are distributed homogeneously within the cell substance, the latter and not readily excretable dyes are distributed in particulate form. A number of indifferently excretable dyes (Edwards, 1926) pile up in the renal cells and remain there for a considerable time before they disappear. Readily excretable dyes are seen microscopically in the cells of the proximal convolutions in maximum amounts only during the period of their maximum elimination in the urine, the cells becoming freer of dye as it disappears from the urine.

It is probable that hitherto undue emphasis has been placed on the presence of dyes in renal cells without appropriate regard for other possible factors such as distribution, appearance (homogeneous or particulate), disappearance, percentage excretion by the kidney and the rate of excretion.

The definiteness with which nephrostomal discs present on the ventral surface of the kidney of Necturus are stained with toluidine blue served to focus attention on them. Camera lucida tracings of a nephrostomal tubule with its nephrostome thickly bordered by long and stout flagella are easily made. These structures are more conspicuous and elaborate than is indicated in the figures of Chase (1923), although his description of them is adequate.

While this investigation does not deal with the functional significance of nephrostomes, it seems, when the literature dealing with their occurrence, structure and function is reviewed, that considerable importance might attach to them. The results presented here indicate clearly that none of the dyes used except toluidine blue is absorbed form the lumina of the proximal or distal tubules. Therefore it would be interesting despite the fact that dyes are not absorbed, if the proximal tubule should at the same time also prove to be the site of the reabsorption of certain substances. White and Schmitt (1926) present data obtained from experiments on Necturus indicating the site of glucose and chloride absorption in the proximal convoluted portion of the renal tubule. Their evidence shows definitely that the reabsorption of these two substances occurs more particularly

in the neck of the tubule rather than in the proximal convolution proper. It is in the neck, however, that the nephrostomal tubule joins the renal. Some experiments of the writer indicate that the nephrostomal tubule might have influenced the reaction obtained by White and Schmitt whereby the absorption of glucose and chloride is shown. These experiments are reserved for a later publication.

With regard to the results obtained by Dawson, possible objections to them in consequence of the method used, are recognized by him as is made clear in his article by the reference made to it in the last paragraph on page 686.

Hayman (1924) sought to test the validity of opinions concerning secretion or reabsorption which were based on histological pictures made from sections of the kidney after the injection of dyes into the blood stream, by comparing the results obtained by observation of the kidneys of living frogs after intravenous and intracapsular injections. He states that intravenous and intracapsular injections yielded similar staining of the tubule cells in all cases. However, since his results have been published only in abstract, it is not possible to discuss them at present.

SUMMARY

Results have been obtained by injecting certain dyes intraperitoneally and intravascularly in Necturus which show that when these dyes are allowed to get into the lumen of the renal tubule through nephrostomes, they are not absorbed by the cells of any portion of the tubule. When they reach the kidney through the blood stream, the proximal tubules are definitely stained by them. They disappear from the cells of these tubules simultaneously and proportionately as they disappear from the urine. Certain features in the behavior of toluidine blue are noted and reference made to the functional significance of nephrostomal tubules.

Grateful acknowledgment is due Dr. E. K. Marshall, Jr., for assistance in connection with the investigation.

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THE EFFECT OF GLYCINE UPON THE METABOLISM OF ISOLATED PERFUSED MUSCLE

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Received for publication October 14, 1926

Since the early observations of Sczelkow (1862) and of Ludwig and Schmidt (1868), a large number of researches have been carried out upon the gaseous exchanges of isolated perfused muscle. The literature of this extensive subject has been reviewed by Barcroft (1908) and by Verzár (1916), and it is not our intent to enter into a resumé of the preceding work, save as it bears a pertinent relation to our own. For the most part, the investigations have had as their goal a study of the effects of temperature, of tonus and contraction, of changes in blood flow and blood pressure, etc., and little attention has been paid to the effect upon the metabolism of adding utilizable foodstuffs to the blood streaming through the tissue.

That the metabolism of foodstuffs within the organism may exert a profound influence upon the heat production and oxygen consumption of the intact animal has been abundantly proved. Both fats and carbohydrates, when ingested, may cause an appreciable increase in the total resting metabolism. But protein and certain of the constituent amino acids of protein (namely, glycine and alanine) have been shown to be stimulators of oxidation *par excellence*, mainly through the investigations of Rubner and Lusk. The evidence is clear that when an amino acid, such as glycine, increases the heat production of an animal, it does so not by its own combustion, with the evolution of "extra" heat, but by an actual spurring of the body cells to increased activity. This was established by Lusk (1915) when he showed that the specific dynamic action of glycine was as demonstrable in a phlorhizinized as in a normal animal.

The seat of this stimulating action in the body, however, remains obscure. There is ample negative evidence that it is not in the gastrointestinal tract, and the fact that glycine, when injected parenterally (Krzywanek, 1923; Weiss and Rapport, 1924) can still increase the heat production, lends positive confirmation to this conception. We are still at a loss, nevertheless, to know *1*, whether this peculiar susceptibility of cellular oxidation to the stimulating action of glycine is widespread throughout the organism; *2*, whether its control is central and dependent

upon the integrity of the animal as a whole, or peripheral and local. Evidence upon these points is conspicuous by its meagreness. In a recent paper, Baker and Carrel (1926) have shown that while amino acids do not promote fibroblast growth, they do stimulate cell migration and possibly multiplication, indicating an apparent activation of cell metabolism. On the other hand, Lusanna (1911) studying the respiration of minced liver and muscle, found that alanine had no effect on the CO_2 production and O_2 consumption, while glycine (as well as other amino acids) in a concentration of 0.08 per cent, actually had a depressant effect. Freise (1913), in the course of an investigation of respiration in the perfused liver, made an isolated observation on the effect of adding glycine to the perfusing fluid and found that the CO_2 production was 95.38 mgm. per kgm. per minute, as compared with an average of 96.96 mgm. in the control livers. The use of CO_2 production under these circumstances as an index of oxidative exchanges is in itself open to grave criticism, but when it is further noted that the average of the control readings is arrived at from figures ranging from 54.74 mgm. to 192.48 mgm. per kgm. per minute, it is obvious that the experiment is necessarily inconclusive, even as regards the liver.

It has been our purpose in the present research to study the questions advanced above, using the technique of the isolated perfused muscle. The evidence that we have obtained indicates that glycine exerts a powerful stimulating effect on the oxidations within the muscle cells, even when the tissue is entirely removed from the body. It follows as a corollary that the specific dynamic action of glycine can be controlled peripherally. It is true that in addition to this local mechanism, a central influence (e.g., through nervous channels) may be admitted as a possibility, but it need not be postulated in the absence of specific proof.

TECHNIQUE. In perfusing the dog's hind leg, it was necessary, first of all, to conserve the free entrance of blood to the muscles, and particularly to allow a free venous return; and secondly, to prevent bleeding. We accomplished these results by means of the following operation. With the animal under light ether anesthesia[1] we made a circular incision through the skin midway up the thigh, cut through and ligatured both ends of all of the thigh muscles (leaving the lower stumps as short as possible); isolated the femoral artery and vein, tying all branches to the thigh muscles below the profunda; tied off the saphenous artery and vein near their junction with the femoral vessels, and ligatured every observable bleeding point in the fascia and cut skin of the distal portion of the leg.

[1] We used ether anesthesia lest the possible depressant effects of a non-volatile anesthetic should persist in the perfused preparation. In a recent communication, published since this work was done, Guttmacher and Weiss (1926) have observed the abolition of specific dynamic action in an animal under urethane.

This last, while laborious, is essential if bleeding is to be prevented, or at least reduced to an occasional and inconsequential oozing. We then sawed through the femur at a point about one inch above the knee, and after scraping out the marrow, stuffed the cavity of the distal stump tightly with absorbent cotton. As an additional precaution against bleeding, we lightly touched the lower ends of the cut thigh muscles, and the exposed skin and fascia, with a cautery. The operation has these advantages: that the perfused muscles are not handled, that they are left intact with skin covering, and that they show no edema even after perfusion for as long as 5½ hours. It may be argued that tissues other than muscle are being perfused. For our purposes, this is not important, as the metabolism of the skin, fat and bone in this region may be considered as constant and negligible. We will assume this to be true, and hereafter regard the metabolism of our preparation as being that of the muscles contained therein.

While the operation, which usually consumed about 1½ hours, was being completed, blood which had been obtained from another dog the same morning, defibrinated, and kept on ice, was introduced into the perfusion apparatus. The latter was constructed by Doctors Katz and Banus for another purpose, and will be described in more detail by them in a forthcoming paper. It consisted in the first place of a pump—a rubber bulb, rhythmically compressed, and arranged with valves to throw the blood through a worm in a water bath into the muscle. The venous return went to an artificial "lung" (a large glass bottle) where, dripping against a revolving vertical slotted rod, it was finely sprayed against the sides of the bottle, and flowed in a thin film into the reservoir beneath, whence it passed to the pump. The bottle, which had a capacity of 4 liters, did not at any time contain more than 600 cc. of blood. Air was circulated through this "lung," and enough CO_2 was added from a tank so that the "expired" air could be held at a constant CO_2 tension, throughout a given experiment, of from 35 to 40 mm. Hg.

Practically the whole apparatus was enclosed in a well insulated box, the temperature of which was controlled by a thermostat. It was, of course, essential for comparable metabolism results that the temperature of the "arterial" blood should be kept constant. The greatest deviation in any experiment was 0.5°; for the most part, the variation was not more than 0.2°, and this source of error, therefore, could properly be disregarded.

The mean "arterial" blood pressure was measured by a mercury manometer. We endeavored as far as possible, consistent with an adequate blood flow, to keep this pressure constant. We felt safe, on establishing blood flow, in permitting the blood pressure to reach a level anywhere between about 60 mm. and 120 mm. Hg. As a rule, it was between 60

and 90 mm. Having reached this level, there was very little alteration in a single experiment. We would have felt suspicious of a rising blood pressure, as indicating a blocking of the venous outflow.

In the "vein" between the muscle and the "lung," a calibrated horizontal side tube was introduced to measure blood flow. When such a measurement was desired, the tube was opened to the air by turning a stopcock, which automatically made a contact through a magnet, shutting off the return of blood to the "lung." The flow per minute was determined by observing the time necessary for the tube to fill to the 2 cc. mark.

We determined the metabolism by taking simultaneous samples of arterial and venous blood at stated intervals (usually 1 hour apart) and analyzing for CO_2 and O_2. These samples were withdrawn from the rubber tubing of the "artery" and "vein" by syringes, the needles of which were filled with paraffin oil. The samples thus obtained were transferred to and preserved under oil in test tubes that were immersed in cracked ice. The CO_2 and O_2 content were determined by the method of Van Slyke and Neill (1924). Our earliest samples were not taken until about 1 hour after the perfusion of the muscle was begun. We felt that it was advisable to allow this length of time for adjustment of temperature, blood flow, blood pressure and CO_2 tension in the "lung" to constant levels.

Under no circumstances did we take a sample of blood unless these factors had been constant for at least 15 minutes, in order that the gas analysis might depict as nearly as possible the true gaseous exchange of the tissues at that time. This precaution was particularly necessary in regard to the blood flow.

The perfusion lasted from 4 to $5\frac{1}{2}$ hours. The muscles, at the end of that time, always appeared normal to gross inspection. Their color was good and there was no apparent edema, a point to which we have already alluded. We observed, however, a tendency for the arterial blood to become darker in color as the experiment continued, nor was this necessarily accompanied by an appreciable decrease in the O_2 content. We are at a loss to account for this phenomenon. It may be a function of methemoglobin production. This, however, is purely speculative.

EXPERIMENTAL RESULTS. *1. The O_2 consumption of resting muscle:* A consumption of oxygen as low as 0.0013 cc. per gram per minute has been observed by Rubner (1885) in mammalian muscle. Other investigators have noted as high as 0.0096 cc., as will be seen in the accompanying table. Our results are of the same order of magnitude as those of earlier investigators. We have seen, as have others, considerable differences in the taking up of oxygen by different muscles—a variation of from 0.0024 to 0.0093 cc. per gram per minute. These figures refer to the earliest

readings in a particular muscle, that is, those taken about 1 hour after the perfusion was begun.

V. Frey (1885) has called attention to the drop in the O_2 consumption of a perfused muscle as the perfusion continues, observing that it is particularly in evidence when the temperature is high (36° to 38°C.). This probably accounts in part for the lowness of the O_2 consumption found by Rubner (1885), for he averages results obtained earlier and later in the same experiment. He himself observes that O_2 consumption decreases with time. We have observed the same phenomenon (fig. 1). In the muscles perfused with whole blood there was a steady lowering of the metabolism, so that at the end of 4 hours the O_2 consumption was from 15 per cent to 50 per cent below the original, the average fall at this time amounting to about 36 per cent. We are obliged to regard this as the reaction of a dying muscle, in which the oxidative processes are gradually slowing up. We observed, in seven experiments, only one exception to

TABLE 1

O_2 Consumption in resting mammalian muscle

AUTHOR	ANIMAL	O_2 PER GRAM PER MINUTE
		cc.
V. Frey (1885)	Dog	0.0029
Rubner (1885)	Dog	0.0013
Chauveau and Kaufmann (1886)	Horse	0.0028–0.0079
Verzár (1912)	Cat	0.0045
Freund and Janssen (1923)	Cat	0.0050–0.0096
Rapport and Katz	Dog	0.0024–0.0093

this rule. In that case the metabolism was, at 4 hours 10 minutes after the beginning of perfusion, 8.5 per cent higher than the original value, whence it dropped to −10.9 per cent at the end of 5 hours, 50 minutes. In one of the control experiments, the animal died before we removed the muscle from the body. In this instance, the decline in oxygen consumption was more marked than in any of the other muscles, reaching −56.6 per cent at the end of 3 hours. We shall return to a consideration of this experiment later. The detailed data in regard to these observations are given in table 2.

2. The effect of adding glycine to the perfusing blood: Van Slyke and Meyer (1913) and Wishart (1915) have shown that skeletal muscle can take up amino acids from the circulating blood, and Van Slyke's evidence indicates that these acids are not heaped up in the tissue, but are either oxidized or synthesized into protein almost as rapidly as they are taken up.

In 4 experiments, 5 grams glycine (Kahlbaum) in 25 cc. water were injected into the venous blood, about 1 hour after the perfusion was begun,

and immediately after the taking of the first sample for analysis. The glycine was thoroughly mixed with the blood in the "lung," and the perfusion continued as before.

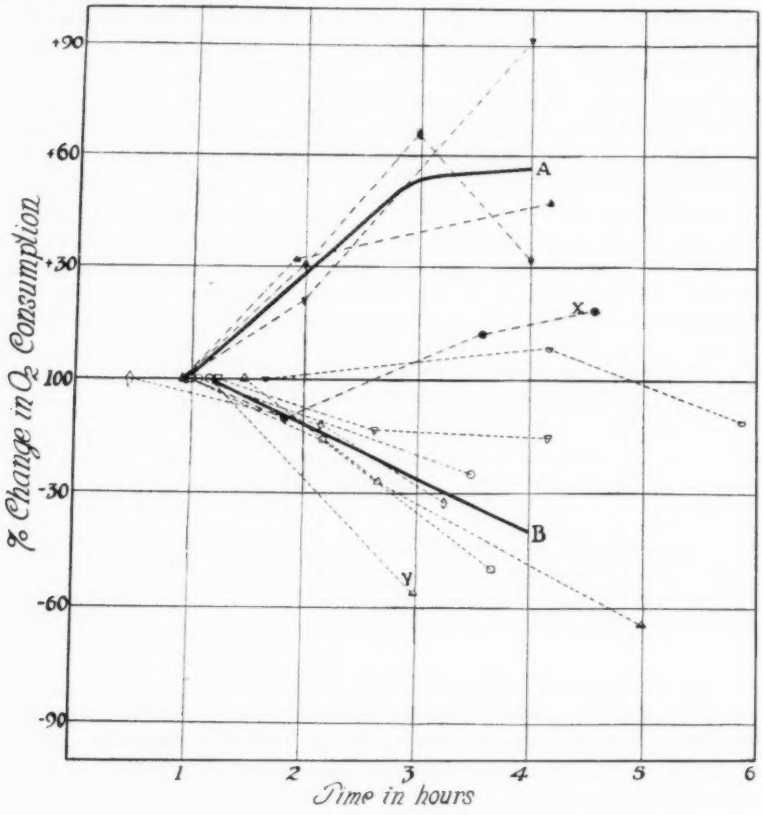

Fig 1. The oxygen consumption of the isolated perfused leg muscles of the dog. Dash lines represent the consumption in muscles perfused with whole blood. The heavy line *B* is the average of these results. Dot-and-dash lines represent the consumption in muscles perfused with whole blood to which glycine has been added. The heavy line *A* is the average of the latter observations. The curves *X* and *Y* were obtained from muscles removed from the body within 5 minutes after the death of the animal.

The contrast between the curve of O_2 consumption in these experiments and that obtained in the controls is striking (fig. 1 and table 3). Instead of the steady fall in the utilization of O_2 seen in the latter, there was in

TABLE 2

*Muscles perfused with whole blood**

DATE	EXPERIMENT NUMBER	WEIGHT OF MUSCLES	BLOOD PRESSURE	TEMPERATURE	TIME AFTER PERFUSION BEGUN		O_2 CONTENT ARTERIAL BLOOD	CO_2 CONTENT ARTERIAL BLOOD	CO_2 PRODUCTION PER GRAM PER MINUTE	O_2 CONSUMPTION PER GRAM PER MINUTE	R.Q.	CHANGE IN O_2 CONSUMPTION	REMARKS
		grams	*mm. Hg.*		*hours*	*minutes*	*volumes per cent*	*volumes per cent*	*cc.*	*cc.*		*per cent*	
July 20	2	?	105	37.5	1	5	16.85	28.84					Muscles not weighed:
			105	37.5	3	30	15.46	31.90				−24.6	O_2 consumption per min. at 1 hour 5 minutes = 0.4329 cc.
July 21	3	93	78	36.8		27	15.46	32.67	0.0097	0.0093	1.04		
			85	36.6	2	10	14.22	36.58	0.0025	0.0082	0.31	−12.3	O_2 consumption per min. at three hours 30 minutes = 0.3352 cc.†
			75	37.2	3	17	12.81	37.52	0.0050	0.0063	0.80	−32.5	
July 22	4	97	90	37.2	1	28	17.35	30.03	0.0029	0.0083	0.35		
			90	37.4	2	40	13.76	31.49	0.0015	0.0060	0.25	−27.7	
			90	37.2	5	00	10.76	?		0.0030		−63.8	
July 26	6	88	96	37.2	1	17	16.68	37.75	0.0027	0.0029	0.94		
			92	37.4	2	37	16.01	34.31	0.0022	0.0025	0.86	−13.2	
			96	37.0	4	9	18.27	32.69	0.0006	0.0024	0.26	−15.3	
July 27	7	59	104	36.6	1	10	13.77	35.57	0.0041	0.0070	0.59		Animal dead before muscle was removed from body
			100	36.2	3	00	12.30	34.05	0.0035	0.0030	1.14	−56.6	
July 28	8	59	128	37.5	1	40	19.38	29.92	0.0027	0.0034	0.79		
			125	37.2	4	10	15.90	36.30	0.0020	0.0037	0.55	+8.5	
			120	37.6	5	50	11.30	38.20	0.0035	0.0030	1.16	−10.9	
August 6	13	82	70	36.8	1	00	15.85	32.10		0.0054			
			64	36.8	2	10	14.84	37.90		0.0045		−16.4	
			64	36.8	4	10	12.66	32.18		0.0028		−50.0	

* For blood flow see table 4.

† Data pertain to Exp. 2.

TABLE 3

*Muscles perfused with blood plus glycine (5 gm.)**

DATE	EXPERIMENT NUMBER	WEIGHT OF MUSCLES	BLOOD PRESSURE	TEMPERATURE	TIME AFTER PERFUSION BEGUN		TIME AFTER GLYCINE ADDED		O_2 CONTENT ARTERIAL BLOOD	CO_2 CONTENT ARTERIAL BLOOD	CO_2 PRODUCTION PER GRAM PER MINUTE	O_2 CONSUMPTION PER GRAM PER MINUTE	R Q.	CHANGE IN O_2 CONSUMPTION	REMARKS
		grams	*mm.* Hg.		*hours*	*minutes*	*hours*	*minutes*	*volumes per cent*	*volumes per cent*	*cc.*	*cc.*		*per cent*	
July 30	9	83	70	37.2		57			17.95	32.90	0.0034	0.0066	0.51		Animal died before muscle was removed from body
			70	37.2	1	49		44	16.78	28.19	0.0045	0.0059	0.76	−10.5	
			70	36.8	3	35	2	30	18.11	28.38	0.0048	0.0073	0.65	+11.9	
			70	36.8	4	35	3	30	16.20	27.09	0.0024	0.0078	0.31	+18.8	
August 2	10	79	64	37.2		53			17.95	38.70	0.0024	0.0049	0.49		CO_2 tension in "alveolar" air accidentally very high
			62	37.4	1	55	1	00	10.25	55.41		0.0064		+32.2	
			68	37.4	4	10	3	15	10.11	61.20		0.0071		+47.0	
			68	37.4	5	10	4	15	5.46	101.80		0.0026		−46.2	
August 3	11	110	94	36.8		55			19.42	25.60	0.0014	0.0025	0.55		
			105	37.2	2	00	1	00	17.49	29.43	0.0018	0.0030	0.64	+21.0	
			105	37.0	4	00	3	00	17.56	27.20	0.0015	0.0048	0.31	+91.6	
August 4	12	82	60	37.0		55			18.39	29.42	0.0017	0.0054	0.32		In the last analysis of arterial blood, the Van Slyke pipette was shaken by hand
			64	37.2	2	00	1	00	14.93	24.10	0.0049	0.0070	0.69	+30.7	
			60	37.2	3	00	2	00	14.59	25.50	?	0.0089	?	+65.9	
			60	37.2	4	00	3	00	13.02	25.56	0.0014	0.0071	0.25	+32.1	

* For blood flow, see table 4.

the glycine experiments a definite increase in metabolism. In 3 of these observations this increase was marked, amounting, on the average, to about 25 per cent at the end of an hour, and to over 55 per cent at the end of 2 hours after the injection of the amino acid. In two of these experiments, the O_2 consumption continued to rise, reaching a figure of 91 per cent above the original level at the 4th hour in one case. In experiment 12 there was apparently a fall after the 3rd hour, for the blood analysis of the 4th hour showed an increase in the O_2 of only 32 per cent. In this analysis, the mechanical shaking device of the Van Slyke apparatus broke down, and a part of the shaking had to be done by hand. It is conceivable that this may have introduced an error, although the observer attempted as far as possible to imitate the mechanical shaking.

In one experiment (no. 9) the animal died at an unknown time (less than 5 minutes) before the muscle was removed from the body. In this instance, the first analysis, 44 minutes after glycine was introduced into the system, showed a decrease of 10.5 per cent in the O_2 consumption. Thereafter, however, the O_2 intake rose until, 2 hours later, it was 11.9 per cent, and 3 hours later, 18.8 per cent above the original reading. This curve may be compared with the curve to which we have already alluded, of a muscle removed from a dead body (expt. 7) and perfused with blood containing no glycine. In the latter case, we observed a decline in the consumption of O_2 which is more rapid than in any other muscle perfused similarly. It is noteworthy that while at first the glycine perfused muscle of experiment 9 also showed a tendency toward a dropping metabolism, the effect of the glycine was ultimately to counteract this, and to stimulate the cells to a metabolism actually greater than that associated with their original degree of activity.

It might be argued that the falling O_2 consumption of the muscle when perfused with whole blood was due to the using up of the oxidizable material, such as glucose, and that glycine supplied that lack. This would hardly account for the actual increase of the cell metabolism above the original level when glycine was introduced. In any case, however, and quite apart from the supply of such material in the muscle itself, which is in all probability adequate, it can be shown by a simple calculation that the blood used would furnish more than enough glucose to account for the consumption of oxygen by the tissue. Thus, the combustion of 1 gram of glucose would require 746 cc. O_2. The maximum consumption per minute in any of our experiments was 0.7608 cc. In 4 hours at this rate the consumption would have been 182.59 cc., which would represent about 0.25 gram glucose. We used about 500 cc. blood for perfusion. Assuming the concentration to have been 0.1 per cent, there would have been ample for the needs of the tissue provided sugar alone were burned.

It seems clear, therefore, that glycine exerts a profound influence upon

the metabolism of muscle completely isolated from the rest of the body.[2] This is particularly striking in our experiments, in view of the constant tendency for the muscle perfused with whole blood to show a decreasing metabolism with the passage of time. We are, therefore, confronted by cells whose oxidative power is decreasing, but which, in the presence of a large amount of glycine, are spurred to an activity considerably greater than they were capable of at the beginning of the experiment, and even more in excess of what they might have been expected to betray had not glycine been added.

The specific dynamic action of glycine accordingly affects directly the tissue whose metabolism is thereby stimulated, and does not require the intervention of a central mechanism outside of the tissue. Whether or not this also applies to the stimulating action of other amino acids, as for example alanine, and to that of proteins, is an open question; generalization would be hazardous.

3. The factor of blood flow and the oxygen supply: The important question of the rôle of the oxygen supply to the tissue as a possible limiting factor in oxidations arose in the course of this work. It involved, from our point of view, a consideration of the blood flow and of the O_2 content of the arterial blood. Verzár (1912, 1920) has stated that the less the circulation through a muscle, the less the consumption of O_2 by that muscle. Nakamura (1921) was unable to confirm this. On reducing the blood flow through the leg muscles of the cat from 4.95 to 1.67 cc. per minute (in one case from 7.0 cc. to 0.29 cc.) he found no change in the consumption of oxygen. Moreover, Barcroft and Müller (1911) increased the flow through the submaxillary gland tenfold by means of yohimbin without increasing the amount of O_2 taken up. Nakamura criticizes Verzár's technique, which involved exposure of the muscle. Perhaps an even more important criticism would be the short duration of the experiments. Within 10 or 12 minutes, the blood flow was changed as much as 200 to 300 per cent (in one case 500 per cent in 17 minutes). Under these circumstances, it is questionable whether the blood gas analyses are a true criterion of the muscle metabolism at a given time. Elias (1913) has pointed out that $\frac{1}{2}$ hour is necessary to obtain equilibrium in a perfused muscle.

[2] In making the above statement one factor is overlooked, namely, the perfusing blood itself. The evidence indicates that shed blood has a negligible metabolism, and there is no proof that the blood in the body has a metabolism of an order of magnitude that would make it a factor to be reckoned with. Still more obscure is the possibility of specific dynamic action affecting this doubtful degree of oxidation. We do not, in fact, know whether amino acids can penetrate the red blood cell. Nevertheless, it is possible that a stimulating action on the oxidation of the red cell takes place, and that this effect might have to be deducted from the quantitative results we have given. It is probable, however, that this error is very small, even if it exists.

TABLE 4

Relation of blood flow and O_2 content to O_2 consumption. Muscles perfused with whole blood

EXPERIMENT NUMBER	WEIGHT OF MUSCLE	BLOOD FLOW PER MINUTE	O_2	O_2 CONTENT OF BLOOD FLOWING THROUGH 1 GRAM MUSCLE PER MINUTE	CHANGE IN O_2 CONSUMPTION
	grams	*cc.*	*volumes per cent*	*cc.*	*per cent*
3	93	20	15.46	0.0332	
		26.4	14.22	0.0556	−12.3
		22.2	12.81	0.0284	−32.5
4	97	25.0	17.35	0.0447	
		28.5	13.76	0.0405	−27.7
		25.0	10.76	0.0277	−63.8
6	88	6.7	16.68	0.0127	
		10.0	16.01	0.0182	−13.2
		7.2	18.27	0.0149	−15.3
7	59	12.5	13.77	0.0292	
		9.5	12.30	0.0198	−56.6
8	59	4.4	19.38	0.0145	
		6.7	15.90	0.0181	+8.5
		4.7	11.30	0.0090	−10.9
13	82	9.1	15.85	0.0176	
		11.8	14.84	0.0214	−16.4
		9.5	12.66	0.0147	−50.0
Muscles perfused with whole blood and glycine					
9	83	8.7	17.95	0.0188	
		8.7	16.78	0.0176	−10.5
		11.8	18.11	0.0253	+11.9
		11.8	16.20	0.0230	+18.8
10	79	10.5	17.95	0.0239	
		12.5	10.25	0.0162	+32.2
		10.5	10.11	0.0134	+47.0
		6.9	5.46	0.0047	−46.2
11	110	6.7	19.42	0.0118	
		8.0	17.49	0.0127	+21.0
		8.3	17.56	0.0133	+91.6
12	82	16.7	18.39	0.0375	
		14.3	14.93	0.0260	+30.7
		13.3	14.59	0.0236	+65.9
		13.3	13.02	0.0211	+32.1

It was necessary for us to observe whether our results might be explainable on the basis of a change in blood flow or O_2 tension. Table 4 shows the relation between the O_2 content of blood passing through 1 gram muscle per minute and the O_2 consumption of the tissue. It will be seen that there is no constant relationship. The tendency of the metabolism to fall in the muscles perfused with whole blood was not in any sense a function of the velocity or O_2 content of the blood. In experiment 7, it is true, a single high reading was associated with an increased O_2 content, but in experiments 3, 6 and 13, the metabolism fell in spite of the fact that more oxygen was contained in the blood. Moreover, there was no apparent relationship between the per cent change in O_2 consumption, at a given time in a particular muscle, and the O_2 content of the blood.

In the experiments where glycine was added to the perfusing fluid this lack of correspondence was again evident. Thus, in experiment 12, the metabolism was 65.9 per cent higher than the original level when the blood circulating through 1 gram of muscle per minute held 37 per cent less O_2. Comparable changes in the O_2 content (as in expts. 4 and 12) were accompanied in the control experiments by a fall, in the glycine experiments by a rise in metabolism.

In one observation, the final one of experiment 10, there did seem to be a causal relationship between the drop in the volume flow and O_2 content and the O_2 consumption. Here a steady rise in the metabolism to + 47 per cent was abruptly succeeded by an O_2 consumption of −46.2 per cent. This was accompanied by a fall in the blood flow, and by a reduction of the blood O_2 to 5.46 vols. per cent.

It would be beyond the scope of this paper to review the literature on the subject, but in the intact animal evidence exists to show that a considerable drop in the O_2 content of the blood can take place without affecting the O_2 consumption. Below a critical level, the first reaction of the body is towards an increased metabolism, due probably to heightened respiratory and cardiac activity, and it is only at very low tensions of O_2 in the blood that a definite fall in metabolism occurs. As Verzár (1920) has pointed out, it does not follow that this is true in isolated tissue. Ludwig and Schmidt (1868) showed that a drop in the O_2 of the blood from 15 vols. per cent to 0.62 vol. per cent, is accompanied by a marked diminution in oxygen usage of an isolated tissue. Verzár himself has attempted to show that the metabolism is a function of the O_2 contained in the perfusing blood. At low levels of the latter, his evidence is striking enough, at high levels it is not so clear. For example (p. 244, expt. 4, loc. cit.) a fall in blood O_2 content from 21.6 to 11.7 vols. per cent is accompanied by a decrease in O_2 consumption of less than 10 per cent. When the O_2 content drops to 8.1 per cent, however, the fall in metabolism

is more marked. There is no doubt that if the supply of O_2 be reduced to a point low enough it can act as a limiting factor and lessen oxidation. It is probable that in experiment 10 the critical level was passed, and that glycine no longer stimulated the cell because the lack of O_2 prevented oxidation. Since for our purposes we regarded this as artefact, we have excluded it from the curve in figure 1.

As in the case of O_2, so changes in the CO_2 content of the arterial blood can likewise be ruled out as a factor in affecting the O_2 exchanges, for again no constant relationship is discernible (cf. tables 2 and 3).

4. The respiratory quotient: Perhaps the most striking thing about the respiratory quotient obtained by methods such as the one we adopted is its variability. Rubner (1885) made two observations (at a temperature of 38.2° and 39.5°) and found R.Q.s. of 0.73 and 1.10. At lower temperatures he observed quotients as high as 3.5. Chauveau and Kaufmann (1886) obtained R.Q.s. of 1.3 to 1.7. Hill and Nabarro (1895, 1902) noted variations from 0.7 to 1.8, and Zuntz (1878) in experiments on the tonus of muscle saw quotients of 0.1 to 1.1. In a recent brief note, Himwich and Castle (1926) state that the average of 19 determinations of the R.Q. of perfused preparations was 0.71 ± 0.10.

Like many of the older investigators we have obtained bizarre quotients, ranging from 0.25 to 1.14 (tables 2 and 3). These quotients undoubtedly possess some significance, but their relation to oxidative metabolism is not clear. Rubner (1885) postulated a possible anaerobic production of CO_2, and states that the R.Q.s are unexplainable according to the ordinary oxidation of the foodstuffs. CO_2 production, he says, is affected by other things than O_2 consumption. V. Frey (1885) concluded that the CO_2 content observed in the venous blood was due not only to oxidative metabolism but also to the driving out of CO_2 from the tissue by the production of fixed acids. As Barcroft (1908) points out, every discussion of the relation of the R.Q. to metabolism postulates that the gas content of the muscle remains unchanged. There is certainly no reason to believe, in the case of CO_2, that this is true. It is clear that the individual quotients which we obtained cannot be correlated with the known facts in regard to the combustion of proteins, fats and carbohydrates. By averaging the R.Q.s in certain of the control experiments (table 2) it is possible to obtain results concerning which conclusions *might* be drawn as to oxidations in resting muscle. For example, in experiments 3, 6 and 7 it might be said that fat was being burned. But it is evident that such an average would not be permissible. We are in reality confronted by the fact that the apparent production of CO_2, as measured by blood analyses, is a component of unknown variables, and that deductions in regard to the relation of the R.Q. to the combustion

of foodstuffs or combinations of foodstuffs, in this type of experiment, are of little value.[3]

SUMMARY

1. A preparation is described for the study of the metabolism of an isolated hind leg in the dog, perfused with whole blood from another dog.

2. The arterial and venous blood of the muscles were analyzed for CO_2 and O_2 by the method of Van Slyke and Neill. Care was taken that the temperature, blood flow and blood pressure should be constant for at least fifteen minutes before the taking of any sample for analysis. The CO_2 tension of the air with which the blood was equilibrated in an artificial "lung" was kept the same throughout the experiment.

3. The oxygen consumption of a resting group of muscles perfused with whole blood was between 0.0024 and 0.0093 cc. per gram per minute.

4. The oxygen consumption in muscles perfused in this way gradually diminished, reaching an average level at the end of 4 hours of about 35 per cent below the original metabolism.

5. When 5 grams glycine were added to the perfusing blood the O_2 consumption, instead of falling as in the control muscles, rose markedly, in 3 experiments the rise continuing for more than 3 hours, while in the 4th the metabolism began to fall after the 2nd hour.

6. The increased oxidation after adding glycine is not due to the fact that the control muscles do not have a sufficiency of oxidizable material, a lack which the glycine would supply.

7. The rate of blood flow and the O_2 content of the arterial blood bear no constant relationship to the amount of O_2 consumption. In the control experiments a fall in metabolism may be associated with an increased supply of O_2 brought to the tissue, in the glycine experiments a rise in oxygen consumption may be accompanied by a decreased O_2 transport in the arterial blood. This was true only above a certain critical level, for in one observation a very low blood flow and O_2 content (5.46 vols. per cent) were apparently the cause of a falling metabolism. The O_2 content of the blood flowing through 1 gram muscle per minute in this instance was 0.0047 cc.

8. *It is concluded that glycine acts as a powerful stimulator of the cell metabolism in an isolated perfused group of muscles.*

9. *As a corollary to the above, it is further concluded that the specific dynamic action of glycine is a direct effect upon the cells of the tissue stimulated, and that no central control mechanism outside of the tissue need be postulated.*

[3] We were at pains to keep the CO_2 tension of the air in the artificial lung constant at 35 to 40 mm. Hg. In experiment 10 (table 3) by accident, the tension of CO_2 increased to a high level. In this experiment there was actually less CO_2 in the venous than in the arterial blood after the first observation, indicating an absorption of the gas by the muscle at the high tension existing.

10. The respiratory quotient, as an index of the oxidative metabolism of different foodstuffs, is found in this type of experiment to be totally unreliable. While averaging the results obtained during a single experiment *might* appear in some instances to entail significant conclusions, this is not justifiable, on account of the wide variation in individual readings. The CO_2 of the venous blood coming away from isolated perfused muscles is not a true criterion of oxidation.

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SUSCEPTIBILITY OF ALBINO RATS TO TETANUS TOXIN FOLLOWING ADRENALECTOMY[1]

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Received for publication October 22, 1926

The probability of an antitoxic function is among the earliest theories arising from experimental investigations on the adrenal glands. A number of attempts have been made to prove that certain drugs and bacterial poisons are destroyed through the influence of the adrenals and that in the absence of the glands such poisons exert a much more powerful toxic action. Most of the reports in the literature on this subject are based upon experiments that could not have yielded reliable information.

Numerous papers, dealing with results obtained on adrenalectomised animals, betray a lack of appreciation, on the part of the investigators, of the importance of adequate surgical technique for such experiments and illustrate how easily faulty interpretations can be made and unwarranted conclusions accepted. Such articles would scarcely merit mention were it not for the fact that some recent writers find in them support for their own work. It is, therefore, desirable again to point out, what ought to be perfectly obvious, that these papers contribute little or nothing of value to our knowledge of adrenal function. This has been emphasized in a recent review by Stewart (1924).

Abelous and Langlois (1892) destroyed the adrenals in frogs and in guinea pigs by actual cautery. It would be difficult to be sure that all the adrenal tissue is removed by such a procedure. In guinea pigs they found that the use of anesthesia resulted in death of so many animals, even after ablation of one adrenal, that for a long time they renounced the use of anesthetics. Abelous (1895) undertook to prove an antitoxic influence of the adrenals to atropine. He reported two series of experiments consisting of *two* frogs each. One frog in which both adrenals were destroyed and another in which the left gland was partially cauterized received the same dose of atropine. They died at the same time. In the other two frogs the liver and adrenals were removed in one and the liver only in the other. A dose of atropine was administered to each and death followed in $4\frac{1}{2}$ and $2\frac{3}{4}$ hours after the operation. They are satisfied, from these experiments, that atropine is more toxic after ablation of the adrenals. Langlois and Charrin (1896) were unable to show any difference in sus-

[1] A preliminary report of this work was presented at the meetings of the Federated Societies for Experimental Biology held in Cleveland, December, 1925.

ceptibility between unoperated and *unilaterally* adrenalectomised guinea pigs to virulent cultures of B. pyocyaneus.

Oppenheim (1901) found no difference between *unilaterally* adrenalectomised and unoperated guinea pigs in susceptibility to tetanus toxin and to anthrax. Each series of experiments consisted of two or three operated and a like number of unoperated animals. With pneumo-bacillus of Friedlander he reported a lowered resistance after unilateral adrenalectomy, on the basis of the following experiments: Two operated and two control guinea pigs were injected with a culture of pneumo-bacilli. One operated animal died in 17 hours, the others survived. Twenty days later the remaining three animals were given another dose of the culture; one control died in 20 hours, the other two animals survived. Fifteen days later the remaining two animals (one operated, the other control) received a third dose of the culture; both survived. We believe further comment unnecessary. His conclusion that adrenalectomy *increases* resistance to diphtheria toxin has just as little foundation.

Camus and Porak (1913), working with rabbits, arrived at the perfectly safe conclusion that in partial adrenalectomy the resistance to curare may be *diminished*, *unaltered* or *increased*. In total adrenalectomy it is always diminished during 24 hours following the operation. Of 10 operated rabbits they report only one in which total adrenalectomy was performed; the others were either partially adrenalectomised or the glands injected with fatty acid. An indication of their surgical results can be seen from the two rabbits constituting group 5 of their series. Both animals were partially adrenalectomised; in the evening one died "spontaneously," the other after a small dose of curare.

Marie and Morax (1914) claimed an increased susceptibility to tetanus toxin, after adrenalectomy, *in one guinea pig*, although in another, in which autopsy revealed that adrenalectomy was incomplete, the same dose was inactive. A number of other papers, some quite recent, could be mentioned, but these will suffice to demonstrate the quality of experimental evidence that is convincing to some writers. Such articles can only render suspect the work of anyone who finds in them support.

Rogoff and De Necker (1925) investigated the alleged increase, of 400 to 500 times, in susceptibility of adrenalectomised white rats to morphine and found no foundation whatever for such a claim. A recent writer (Jaffe 1926) seems to take exception to their conclusion. He states, "Rogoff and his co-workers report no significant change in the tolerance to morphine or tetanus toxin following suprarenalectomy, but they tested tolerance to substances which are extremely toxic even to normal rats. Their conclusions may be disputed, particularly in regard to morphine, if Table I of a recent paper (Rogoff and De Necker) is analysed. Of sixty-five animals injected between eight and thirty-six days after suprarenalectomy with doses of this drug ranging from 0.1 to 0.25 mgm. per gram body weight, which is less than the minimum lethal dose for normal rats, forty-four or about 67 per cent were killed."

His criticism is indeed remarkable. It is true that the substances tested are quite toxic to rats, but we can see no object in testing the toxicity of non-toxic substances. We find it difficult to understand why he selects only a little more than half the animals given in the table, to point out that about 67 per cent died following administration of morphine. If one is inclined to do so he could select a group from the table that would show a death rate of more than 80 per cent. But this is unnecessary, for if the percentage death rate following administration of morphine impresses him as strikingly high, why not include the entire table? The results will not vary materially from his figures. Out of 114 adrenalectomised rats that received morphine 72 (about 63 per cent) died. In discussing this question on the basis of percentage death rate, however, we must not overlook, or conceal, the fact, which is quite well

established, that about 50 per cent of all rats die after complete adrenalectomy, even if no poison is administered. In reality, therefore, only about 10 to 15 per cent additional were killed by morphine administered in doses ranging up to the lower limit of the M.L.D. for unoperated rats. It is amazing to find that this does not impress him, and that this writer quotes, without comment or criticism, Lewis' report of an increase in susceptibility to morphine of 400 to 500 times, although about 37 per cent of adrenalectomised rats, published in the table under discussion, survived doses of morphine ranging from 10 to 250 times the dose that is considered by Lewis as fatal.

The statement, made by Jaffe, that Rogoff and collaborators "state that, if tested after having fully recovered from the immediate operative effects, suprarenalectomised rats do not show increased susceptibility to toxins and drugs," is incorrect. We have always had an open mind on the question and would be quite prepared to accept a report based upon convincing experimental evidence. We have, thus far, investigated only morphine and tetanus toxin and are convinced that adrenalectomy does not materially alter the susceptibility in rats to these substances. In regard to morphine, we must repeat that there is no foundation for the claim of an increased susceptibility of 400 to 500 times in adrenalectomised rats, and that Scott (1923) did not confirm Lewis (1921), but if anything has shown the opposite and has added evidence supporting our opinion. Scott's published experiments show no such diminution in the M.L.D. as Lewis claims, and it is astonishing that he should have seen in them confirmation of Lewis' conclusions.

Histological changes have been observed in the adrenals following certain infections or intoxications. These may be important, but it is risky to make physiological deductions from histological evidences alone. Thus, we have seen degenerative changes in cortical cells of fragments of adrenal that have been spared during adrenalectomy. This is to be expected, and since such remnants of adrenal can, by an inexperienced worker, be mistaken for accessory glands it would be misleading to assume that these changes are associated with function of the adrenal tissue.

The eventual onset of a serious intoxication after removal of the adrenal glands has been demonstrated from the recent studies by Rogoff and Stewart (1925, 1926a, 1926b) on adrenalectomised dogs. In such animals the clinical progress, the development of a severe gastro-intestinal upset, changes occurring in the blood, relief of symptoms and prolongation of life by intravenous administration of physiological salt solutions, and the post-mortem findings may be interpreted as evidences of a profound intoxication due to loss of adrenal function. Whether this intoxication may itself be due to the loss of a hormone cannot be settled at present.

We were led to examine the influence of adrenalectomy on the susceptibility to tetanus toxin by the fact that in a large number of animals the loss of the adrenals leads to the same symptoms as are evoked by this toxin. If suppression of adrenal function gives rise to the accumulation of toxic products which poison certain structures, it seemed not improbable that a toxin that acts in a similar manner might be less easily tolerated after adrenalectomy. There has been up to the present time, no satisfactory evidence

of an altered susceptibility to tetanus toxin after adrenalectomy. Marie (1912) reported a neutralising effect, in vitro, of adrenalin on tetanus toxin. Tawara (1921), however, found that this influence was due to acid present

TABLE 1

Bilaterally adrenalectomised rats in which no accessory glands were found post-mortem

NUMBER OF ANIMAL	WEIGHT WHEN RECEIVED	IN LABORATORY BEFORE OPERATION	WEIGHT WHEN OPERATED	WEIGHT WHEN INJECTED	DOSE OF TOXIN ADMINISTERED	TESTED AFTER OPERATION	DIED OR SACRIFICED AFTER OPERATION
	grams	*days*	*grams*	*grams*	*cc.*	*days*	*days*
1041	115.5	3	122.0	123.0	0.1	13	44*
1043	114.5	3	121.6	141.5	0.1	13	44*
1048	127.5	3	133.0	166.5	0.1	13	44*
1049	113.0	3	130.0	174.0	0.1	13	44*
1050	116.5	3	129.8	173.5	0.1	13	44*
1111	124.5	17	177.0	157.5	0.1	4	11
1112	115.5	17	187.0	184.5	0.1	4	32*
1064	114.0	3	118.4	110.0	0.25	13	16
1065	114.5	3	123.0	178.0	0.25	13	44*
1067	112.5	4	120.0	153.0	0.25	24	36
1072	110.0	4	110.0	122.0	0.25	24	33
1073	123.0	4	132.5	166.0	0.25	12	†
1077	115.5	4	133.0	201.0	0.25	24	44*
1088	111.0	4	112.0	110.0	0.25	24	30
1091	120.0	16	177.8	172.5	0.25	5	32*
1097	116.5	16	168.8	144.0	0.25	12	14
1101	125.0	16	190.0	153.0	0.25	12	14
1102	117.5	16	172.0	228.0	0.25	12	29
1103	126.0	16	174.5	187.5	0.25	12	32*
1104	114.5	16	183.0	206.5	0.25	12	32*
1114	115.0	17	190.0	186.0	0.25	4	10
1115	124.0	17	178.0	168.5	0.25	4	15
1116	123.5	17	180.0	162.5	0.25	4	9
1124	117.0	17	190.0	189.5	0.25	11	26
1125	114.0	17	181.5	154.0	0.25	11	14
1084	113.5	4	124.0	190.0	0.5	24	33
1106	117.0	17	172.5	194.0	0.5	11	18
1108	128.5	17	183.5	170.0	0.5	11	16
1109	120.0	17	189.0	194.0	0.5	11	18
1110	113.0	17	164.0	190.0	0.5	11	17
1121	109.0	17	180.5	153.0	0.5	11	16
1123	114.0	17	175.0	146.5	0.375	11	15

* Sacrificed.

† Escaped from cage on 18th day and was killed by a dog.

as a preservative in the adrenalin solutions. It is not very likely that the medulla of the adrenal is very important in this question, for it is well established that the indispensable function of the glands is in the cortex,

since animals live indefinitely, in perfect health and behavior, after the epinephrin secretion of the adrenals has been greatly interfered with or abolished.

We employed albino rats. Thirty-nine unoperated animals were used as controls and to determine the M.L.D. of the toxin. Eighty-six rats were operated; in sixty-seven both adrenals were completely excised, in fourteen

TABLE 2

Rats in which partial adrenalectomy was performed or accessory cortical tissue found post-mortem

NUMBER OF ANIMAL	WEIGHT WHEN RECEIVED	IN LABORATORY BEFORE OPERATION	WEIGHT WHEN OPERATED	WEIGHT WHEN INJECTED	DOSE OF TOXIN ADMINISTERED	TESTED AFTER OPERATION	DIED OR SACRIFICED AFTER OPERATION
	grams	*days*	*grams*	*grams*	*cc.*	*days*	*days*
1045	111.0	3	120.0	148.0	0.1	13	44*
1047	121.0	3	131.5	168.0	0.1	13	44*
1061	111.5	3	115.5	160.0	0.1	13	44*
1095	106.0	16	175.5	171.5	0.1	5	32*
1096	118.0	16	174.8	175.5	0.1	5	32*
1098	126.0	16	174.5	180.0	0.1	5	32*
1113	116.0	17	180.0	183.0	0.1	4	31*
1053	113.0	3	118.5	190.0	0.25	24	44*
1068	121.0	3	130.0	200.0	0.25	24	44*
1063	121.5	3	129.8	183.0	0.25	13	35
1085	113.5	4	132.0	194.0	0.25	24	44*
1090	114.0	4	125.0	166.0	0.25	24	44*
1092	124.5	16	178.0	173.5	0.25	5	31
1093	114.5	16	175.5	156.0	0.25	5	10
1076	116.0	4	125.5	200.0	0.375	24	35
1079	118.5	4	121.0	190.0	0.5	24	31
1107	108.5	17	171.0	140.0	0.5	11	14
1118	115.0	17	165.0	135.0	0.5	11	14
1119	112.0	17	171.5	147.0	0.5	11	16
1120	112.0	17	182.5	208.0	0.5	11	18

* Sacrificed.

In rats 1045, 1085 and 1118 accessory cortical tissue was found; 1096, 1098 and 1113 one adrenal only removed; 1053 and 1068 laparotomy and traumatization performed in the region of the adrenals (included in table for convenience); in all the others a portion of one gland was spared and was usually found more or less hypertrophied at autopsy.

a portion of one gland was purposely spared, in three one adrenal only was excised and in two laparotomy was performed without removing the adrenals. All the surgical operations and post-mortem examinations were performed by one of us (R) and the toxin was prepared, standardised and administered by the other (E), each keeping separate notes on his part of the investigation until the experiments were completed.

The toxin was a dry powder, kept in a brown sealed ampoule in a refrigerator for over a year before being used. Its toxicity was determined in January and again in June and there was no difference in the M.L.D. A stock solution was prepared containing 80 mgm. of the powder dissolved in 100 cc. sterilised physiological saline. For administration, this was further diluted 1 to 10 and the dose used was in each case made up to a volume of 1 cc. with salt solution before injection. In all the animals the toxin was injected intramuscularly, in the inner aspect of the left leg. To determine the M.L.D. doses ranging from 0.1 cc. to 2.0 cc. of this 1:10 dilution were given to rats weighing in the neighborhood of 150 grams, average. We found that all doses of 0.5 cc. and higher were fatal. The

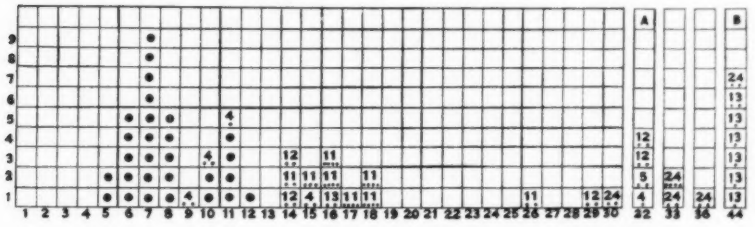

Fig. 1. Distribution of deaths in adrenalectomised rats.

Total number of rats with complete adrenalectomy, 60. Injected with tetanus toxin, 32. Not injected, 28.

Doses of tetanus toxin are indicated by the smaller dots as follows: one dot, 0.1 cc.; two dots, 0.25 cc.; three dots, 0.375 cc.; four dots 0.5 cc. (M.L.D.).

The number within the square indicates the number of days following adrenalectomy in which the toxin was injected.

The animals indicated in columns A and B were sacrificed, 32 and 44 days, respectively, after adrenalectomy.

The numbers along the horizontal axis represent days after adrenalectomy and the vertical indicate the number of rats.

M.L.D. determined at 0.5 cc. caused local paralysis in one day, later arching of the back and general tetanus, followed by death after about 5 to 6 days. Doses of 0.1 cc. and 0.25 cc. caused local paralysis and with the latter dose frequently general tetanus, but the animals recovered.

The technique employed for adrenalectomy in rats is sufficiently described in a previous paper (Rogoff and De Necker, 1925). Of the 125 rats used in this investigation, 102 were obtained from one source of supply, the others were raised in this laboratory. The operated and control animals were kept in a light, well-ventilated room, in individual cages, and in all respects under similar conditions. The diet was the same as described in the above-mentioned paper. Rats thrive and keep in excellent health on this diet and under these conditions, whether unoperated or surviving

the loss of their adrenal glands. This is illustrated in tables 1 and 2 by comparing the weights of the animals when received in the laboratory with their weights at the time of operation and when injected with the toxin.

Our experiments show that there is no significant difference between unoperated rats and rats that were subjected to total or partial adrenalectomy in their tolerance to tetanus toxin. Of the thirty-two operated rats in which post-mortem macroscopic examination failed to reveal the presence of any adrenal tissue (table 1), seven were injected with one-fifth, eighteen with one-half and seven with the full M.L.D. of the tetanus toxin, four to twenty-four days after excision of the adrenals. The distribution of deaths among these animals, together with twenty-eight adrenalectomised rats in which accessory adrenal tissue was not found post-mortem and which did not receive tetanus toxin, is illustrated in figure 1. The

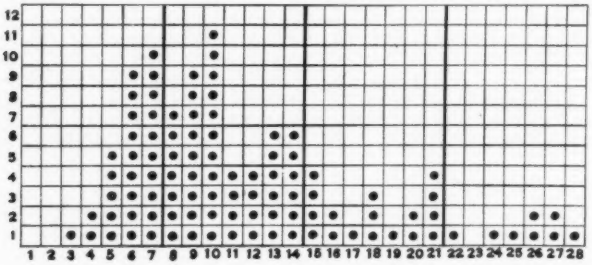

Fig. 2. Distribution of deaths resulting from complete adrenalectomy in albino rats.

The numbers along the horizontal axis represent days after adrenalectomy and the vertical indicate the number of rats.

larger dots represent untreated rats, and the animals injected with the toxin are indicated by the numbers within the squares, each number corresponding to the number of days following adrenalectomy, in which the toxin was injected and representing an animal. The smaller dots below these numbers indicate the dose of toxin administered. One rat is not included in the figure. It got 0.25 cc. of the toxin on the 12th day after adrenalectomy; on the 18th day it escaped from the cage and was killed by a dog.

Comparing this figure with figure 2, which shows the distribution of deaths among ninety-nine adrenalectomised rats (reported by Rogoff and De Necker) in which no adrenal tissue was found post-mortem (macroscopically), it will be seen that, with doses of tetanus toxin up to the M.L.D., there is practically no difference in the usual survival periods of adrenalectomised rats between those injected with the toxin and untreated

animals. Details of the experiments on the thirty-two completely adrenalectomised rats are given in table 1.

In preliminary experiments we administered one-fifth M.L.D. (0.1 cc.) of the toxin to seven rats that were surviving double adrenalectomy. Two were injected on the 28th day, one on the 57th day, one on the 59th day, two on the 60th day and one on the 78th day following the operation. All developed local paralysis but recovered completely and were sacrificed more than two months later. At autopsy, one rat that was injected on the 28th day and one on the 60th day were found to possess accessory cortical tissue, the others showed no macroscopic adrenal tissue. Among animals that survive adrenalectomy longer than about four or five weeks, macroscopic accessory adrenals are more commonly found. To test the statements in the literature, regarding tolerance to poisons in partially adrenalectomized animals, we included a number of animals that were subjected to incomplete removal of the glands. Since we found no difference in susceptibility to tetanus toxin after complete adrenalectomy it could not, of course, be expected that these animals would show anything else. But they will serve very well as operated controls. Three were adrenalectomised on one side only, in twelve a portion of one adrenal gland was spared and in two laparotomy was performed and the region of the adrenals traumatized but the glands were not excised. Three rats, in which double adrenalectomy was performed but in which accessory adrenals were found post-mortem, are included with these animals in table 2. Tetanus toxin was administered in these animals, as in the completely adrenalectomised rats, in doses of one-fifth to the full M.L.D. It will be seen from table 2 that all the rats survived one-fifth of the M.L.D. of toxin and of seven animals that received one-half M.L.D. only one died within the usual killing time of the toxin.

The shortest survival periods, following adrenalectomy are seen among the animals that lost weight before or after the operation or that had failed to gain before being operated. This is true whether or not they were injected with tetanus toxin. Of the animals that continued to gain in weight after removal of the adrenals, many of those that were injected with the toxin, in doses below the M.L.D., recovered from the effects of the toxin, some of them dying later (at about the same time as untreated adrenalectomised rats), others living till sacrificed on the thirty-second and forty-fourth days.

From the foregoing, it will be seen that there is no foundation for the statements found in the literature that there is an increased susceptibility to tetanus toxin following partial or complete adrenalectomy. Such statements are chiefly the result of poorly executed experiments, inadequate controls and a failure to recognize some of the important factors (surgical) which contribute to early death of operated animals.

SUMMARY

The susceptibility to tetanus toxin after adrenalectomy, in albino rats, was investigated. One hundred twenty-five rats were used. Thirty-nine unoperated animals were employed as controls and to determine the M.L.D. of the toxin; eighty-six were operated. In sixty-seven both adrenals were completely excised, in fourteen a portion of one gland was purposely spared, in three only one adrenal was removed and in two laparotomy was performed without removing the glands.

Tetanus toxin was administered in doses ranging from one-fifth to the full M.L.D., four to twenty-four days following excision of the adrenals. To eliminate possible personal factors, the toxin was prepared, standardised and administered by one of us (E) and the surgical operations and post-mortem examinations by the other (R), each keeping separate notes on his part of the investigation until the experiments were entirely completed.

We conclude that there is no foundation for the statements found in the literature that partial or complete adrenalectomy results in an increased susceptibility to tetanus toxin.

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THE EFFECT OF THE EXPERIMENTAL ADMINISTRATION OF EXCESSIVE AMOUNTS OF WATER

I. On the Volume and Concentration of the Blood

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Received for publication October 25, 1926

The blood is as remarkable for its constancy of composition as for its complexity. The importance of its ability to maintain the constant internal environment, the "milieu interieur" of Claude Bernard, so essential to the normal and uniform functional activity of the tissues, has long been recognized. In a volume of fluid continuously renewed and replaced it is indeed remarkable that the great variety of dissolved substances should deviate so slightly under the widely varying conditions involved in the daily cycle of food intake and of removal of waste.

An enormous amount of investigation has been carried out in an effort to determine the different mechanisms governing the composition of the blood. Particular emphasis has been placed on the control of the water, protein, and salt content, the reaction, the regulation of the amino-acids, urea, and other nitrogenous constituents, the sugar and fat content, and the gas exchange.

The constancy of the volume of the blood has been accepted, but data relative to volume changes in the living animal have been difficult to obtain. The recent development of simple methods for determining the quantity of the circulating blood or plasma affords an opportunity for the consideration of changes in the volume as well as in the composition of the blood.

Changes in the water content of the blood have been studied from many different angles. Keith (1919) and more recently Cannon (1923), among others, have emphasized the changes in the volume and composition of the circulating blood associated with surgical or traumatic shock. Marriot (1923) and Utheim (1920) have studied the effects of the similar changes observed in connection with anhydremia and severe nutritional disturbance in infants. Keith (1924) has also studied in detail the dehydration experimentally produced by the intravenous injection of a strong solution of sugar. By this means he was able within a few hours to produce a loss of from 7 to 10 per cent of the animal's entire store of water, together

with a reduction of 40 per cent in the total volume of the blood. With such great reduction in the volume of the circulating fluid the mechanical effects on the circulation are of paramount importance.

The presence of a true hydremia in cases of edema of renal origin has been postulated since the time of Bright. The concentration of proteins in the plasma may be low in such cases, but the recent studies of Linder, Lundsgaard, van Slyke and Stillman (1924), and of Brown and Rowntree (1925), have shown that this is due to a decrease in the absolute as well as the relative amount of plasma proteins, and not to an increase in the plasma volume.

There is a temporary increase in the blood volume after the intravenous injection of a physiologic solution of sodium chlorid. Plesch (1909) has made this the basis of his method of determining the blood volume. This increase, however, is transitory, for the injected fluid leaves the blood stream with extreme rapidity. Bogert, Underhill and Mendel (1916) found that in rabbits the normal blood volume is restored within thirty-five minutes following the intravenous injection of an equal volume of sodium chlorid solution. In dogs the restoration of the blood volume occurred within forty-five minutes. However, if an excessive amount of such solution be given intravenously (Cohnheim and Lichtheim, 1877, injected amounts up to 92 per cent of the initial body weight of the animal) the protective devices of the body may fail and hydremic plethora as described by Cohnheim and Lichtheim, Magnus (1899), Plesch, and Bayliss and Starling (1894) develops. This condition of hydremic plethora can be produced by the intravenous injection of physiologic sodium chlorid solution only, since distilled water produces rapid and fatal hemolysis.

Changes in the blood produced by the oral administration of water have not been consistently demonstrated. Jones, in 1887, observed a decrease in the specific gravity of the blood following the drinking of 700 cc. of water. Strauss and Chajes (1904) found a slight decrease in the refractive index of the serum after the ingestion of one liter of water. Engel and Scharl (1906), on the other hand, gave from 1000 to 1400 cc. of mineral water without decreasing the refractive index of the serum. MacCallum and Benson (1909) took quantities of water up to 2000 cc. without causing demonstrable changes in the erythrocyte count. Robertson and Bock (1919) found that forcing the intake of fluids after a severe hemorrhage hastened the restoration of the normal blood volume, while Underhill and Kapsinow (1922) and Underhill and Roth (1922) have obtained similar results when the blood has been concentrated in consequence of deprivation of water. Veil (1916) reported the effect of the intake of 6250 cc. of water daily for eleven days with an otherwise constant diet. He found a slight reduction both in the hemoglobin content of the blood

and in the serum protein. Bakwin (1922) gave normal infants from 30 to 35 cc. of water for each kilogram of body weight, and in ten of twelve experiments there was a reduction in the refractive index of the serum. Perhaps the most striking experiments in this connection were those of Haldane and Priestley (1915–1916). They gave 5500 cc. of water in a period of four hours and secured marked diuresis, the urinary output at one time being at a rate of 2500 cc. an hour. Even with this great water transport they were unable to demonstrate any associated change in the hemoglobin content of the blood. They concluded that in normal persons the water content of the blood is practically constant and cannot be affected by drinking water. The chemical control exercised by the kidneys they consider comparable in sensitivity to the response of the respiratory center to minute changes in carbon dioxid concentration. This experiment was later confirmed by Adolph (1921).

The quantities of water taken by Haldane and Priestley represent maximal amounts for a normal person. Miller and Williams (1921) reported the development of untoward symptoms in patients with hypertension and nephritis following the ingestion of large amounts of water, 10 liters a day. Weir, Larson and Rowntree (1922) reported similar symptoms and the development of convulsions in patients with diabetes insipidus who maintained a high intake of water after the polyuria had been controlled by the use of pituitary extract. Rowntree (1923) has particularly studied the effects on animals of the forced ingestion of excessive amounts of water. Marked toxic manifestations were regularly produced in all laboratory animals studied by him (dogs, cats, rabbits, guinea pigs) and described under the name of water intoxication.

In the present experiments water has been given to dogs in amounts greatly in excess of any physiologic need. The response of the body to quantities greater than can adequately be cared for by the normal physiologic mechanism has been studied. Only the effects on the blood as a whole are reported here.

The experimental procedure. The conditions of the present experiments were those described by Rowntree. Distilled water in quantities of 5 per cent of the body weight was given to dogs by stomach tube at half-hourly intervals. The day preceding the experiment water but no food was allowed. Blood samples were taken from the jugular vein at the beginning, at three-hour intervals, and at the termination of the experiment. Half-centimeter quantities were drawn hourly for the hemoglobin estimations. The administration of water was continued for from five to eight hours, depending on the condition of the animal. A final sample of blood was taken twenty-four hours after the initial specimen and before the dog was fed. A large number of blood constituents were studied, but the number of determinations in any one experiment was

TABLE 1

Changes in blood concentration following the administration of excessive amounts of water

Dog 3. March 10, 1923

TIME	WATER ADMINISTERED	TOTAL	WEIGHT	BLOOD	SERUM						REMARKS
				Hemoglobin	Refractive index, 20°C.	Relative viscosity, 20°C.	Albumin-globulin ratio	Surface tension. Scale reading	Specific conductivity, 1×10^{-4}, 25°C.	Δ	
	cc.	*cc.*	*kgm.*	*per cent*							
Normal			11.0	100.0	1.3473	1.74	28:72	87	109.8	0.629	
8:30	550										
9:00	550										
1 hour		1,100		94.4							
9:30	550										
10:00	550										
2 hours		2,200		88.6							
10:30	550										Vomited irregularly
11:00	550										
3 hours		3,300	11.8		1.3475	1.72	32:68	89	104.2	0.623	
11:30	550										Vomited irregularly
12:00	550										
4 hours		4,400		89.0							
12:30	550										Salivated; vomited
1:00											
5 hours		4,950									
1:30											Salivated; vomited
2:00	550										
6 hours		5,500	12.8	82.6	1.3456	1.63	25:75	90	93.4	0.577	
2:30	550										Salivated; vomited
3:00	550										
7 hours		6,600		84.1							
3:30	550										Convulsions
4:00		7,150									
8 hours			12.3	84.1	1.3447	1.55	28:72	91	95.1	0.570	
24 hours					1.3465	1.66	30:70	86	106.0	0.613	Recovered

limited so that not more than 50 cc. of blood was taken during its course. Large animals were used and the quantity removed was not sufficiently great of itself to cause changes in the composition of the blood. Control experiments were made at other times, in which the same animals were

TABLE 2

Changes in blood concentration following the administration of excessive amounts of water

Dog 4. March 14, 1923

TIME	WATER ADMINISTERED	TOTAL	WEIGHT	BLOOD	SERUM						REMARKS
				Hemoglobin	Refractive index, 20°C.	Relative viscosity, 20°C.	Surface tension. Scale reading	Specific conductivity, 1×10^{-4}, 25°C.	Δ	Chlorids, NaCl	
	cc.	*cc.*	*kgm.*	*per cent*						*mgm. per cent*	
Normal			11.0	100.0	1.3472	1.71	90	106.2	0.594	631	
8:30	550										
9:00	550										
1 hour		1,100		92.0							
9:30	550										
10:00	550										
10:30	550										
11:00	550										
3 hours		3,300		86.7	1.3451	1.56	92	90.6	0.538	476	
11:30	550										Salivated;
12:00	550										vomited
4 hours		4,400		75.8							
12:30	550										Urinated;
1:00	550										vomited
5 hours		5,500	11.1	78.2	1.3450	1.56	94	84.3	0.520	476	Convulsion
24 hours				93.2	1.3470	1.70	90	102.5	0.610	586	Recovered

used and a volume of blood was drawn equal to, or double, that taken in the water experiments.

ANALYTIC METHODS. Hemoglobin estimations were made by using the initial specimen as a standard and comparing subsequent samples with it in a colorimeter of the Duboscq type. The hemoglobin is reported in

terms of the relative, rather than the absolute, amount of hemoglobin present. The serum proteins were determined by the refractometric method with a Pulfrich refractometer. The protein concentration was calculated from the index of Neuhausen and Rioch (1923) rather than that of Reiss (1913). A constant correction was made for the non-protein constituents of the serum.[1] The relative viscosity of the serum was estimated by the instrument of Hess.[2] The surface tension of the serum was measured by the instrument of du Noüy (1922). The dye method of Keith, Rowntree and Geraghty (1915) was used for the estimation of the plasma volume and the whole blood volume. Normal determinations

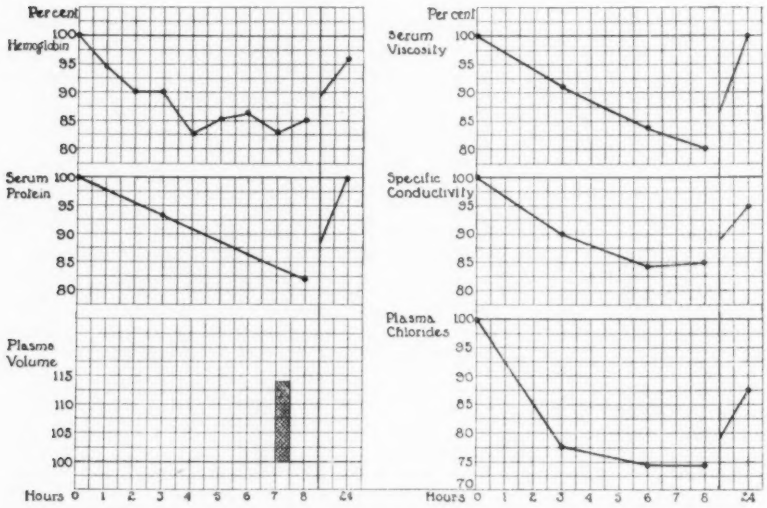

Fig. 1. Chart showing the average change in various constituents of the blood in percentage of the initial value.

were made at intervals of several days before the administration of the water, and the average of two or three concordant determinations was taken as representing the mean normal plasma volume. Plasma volumes were determined at the end of the experiment. The hemoglobin was

[1] It seems probable that the non-protein constituents of the serum decrease slightly during these experiments. The value of this decrease was not determined; however, such change if present would not introduce an error greater than 1 per cent in the value for the serum proteins and so may be disregarded.

[2] Hemolysis causes a marked increase in the viscosity of serum, hence hemolyzed specimens were discarded. Hemolysis, when present, was found in individual tubes only, and was due to manipulation of the blood sample. There was no evidence of intravascular hemolysis in any of the experiments.

measured at the beginning of these experiments, but no other blood samples were taken. These experiments, therefore, also serve as a control to the effect of the blood samples taken in connection with the chemical studies.

EXPERIMENTAL DATA. The experimental results are presented chiefly in tabular form. Protocols of two typical experiments are given in tables 1 and 2 and in figure 1. In tables 3, 4, 5 and 6, the changes in each of the constituents of the blood are shown in more detail.

Hemoglobin (table 3). All the constituents of the blood studied show striking changes during the course of the experiments. The hemoglobin content of the blood is readily determined with accuracy, and has been so extensively used as an index to changes in the concentration of the blood that it is presented first. Many observers have reported on diurnal variations in the hemoglobin content of the blood (Dreyer, Bazett and Pierce, 1920; Rabinovitch, 1923–1924; Chanutin, Smith and Mendel, 1924). These variations are well shown in the control animals studied by us. Under the present experimental conditions these variations appeared irregularly, and in a series of animals the average was but little affected by the changes in the different individuals.

Following the administration of water by mouth there is a rapid and progressive fall in the hemoglobin concentration in the blood. This is most marked during the first two hours, but persists if the forced administration of water is continued. The maximal decrease in the hemoglobin varies from 10 to 20 per cent in the different experiments, but is about 15 per cent on the average. Very little increase was noted after the fourth hour, although in some instances the experiments were continued for eight hours. The recovery was rapid and complete although not followed in detail. Within twenty-four hours the hemoglobin content of the blood returned on the average to 96 per cent of the original value.

It is generally accepted that experimentally induced changes in the specific gravity and total solids of the whole blood are accurately paralleled by changes in the hemoglobin content. These physical properties of the blood were not studied extensively, but individual experiments indicated that there was a definite decrease in both. The hematocrit reading either was unchanged or was decreased slightly.

Serum protein (table 4). The total quantity of serum proteins decreased from an average of 6.8 per cent to 5.9 per cent during the experiment. There was complete recovery by the following day. This represents a decrease of about 15 per cent in the amount of proteins normally present.

The relative viscosity of the serum (table 5) changed on the average from 1.79 to 1.65 during the experiment. Recovery was complete by the following day. If only the increase over the viscosity of water, which

TABLE 3

Changes in the relative hemoglobin concentration following the administration of excessive amounts of water

	NORMAL VALUES	TIME IN HOURS								
		1	2	3	4	5	6	7	8	24
		Per cent of normal value								
Control 1	100	100	89	87	90	96	100	100	102	100
Control 2	100	102	100	110	100	102	115	106	100	111
Control 3	100	95	105	100	103		98	95		101
Control 4	100	100	98	102	101	101	99	99	101	95
Average	100	99	98	100	98	100	103	100	101	102
Experiment 1	100		85	85		83	81			95
Experiment 2	100	100	88	88	85	83	88	83		
Experiment 3	100	94	89		89		83	84	84	
Experiment 4	100	92		87	76	78				93
Experiment 5	100	90		81	79		78	79	78	99
Experiment 6	100	98	98	98		96	94	89	90	97
Experiment 7	100	89	91	90		89	83	82	81	94
Experiment 8	100			98			95		90	102
Experiment 9	100			95			90			92
Average	100	94	90	90	82	86	86	83	85	96

TABLE 4

Changes in the serum protein following the administration of excessive amounts of water

	NORMAL VALUES	TIME IN HOURS			
		3	6	8	24
		Grams for each 100 cc.			
Control 1	7.10		7.35		7.35
Control 2	6.30		5.75	6.45	6.15
Control 3	6.80	7.03	6.77	6.98	6.77
Control 4	6.60	6.97	6.68	6.13	6.18
Average	6.70	7.00	6.63	6.52	6.51
Per cent of normal value	100	104	99	97	99
Experiment 1	6.70	5.35	5.15		6.45
Experiment 2	7.15	6.55	6.45		7.50
Experiment 3	6.30	6.45	5.45	5.10	5.95
Experiment 4	6.30	5.15	5.15		6.15
Experiment 5	7.10	6.50	6.30		6.50
Experiment 7	6.90	7.40	5.80	5.95	7.35
Experiment 10	7.30	7.20	6.75		
Average	6.81	6.37	5.87	5.53	6.75
Per cent of normal value	100	94	87	82	99

is a measure of the amount of serum proteins, is considered, then the percentage change in the serum protein is similar in magnitude to that indicated by the change in the refractive index. The constancy in the relationship between these two readings would indicate that there was no disturbance in the albumin-globulin ratio of the serum during the course of the experiments.

The determinations of variation in the surface tension of serum were

TABLE 5

Changes in the viscosity of the serum following the administration of excessive amounts of water

	NORMAL VALUES	TIME IN HOURS			
		3	6	8	24
		Relative viscosity			
Control 1	1.90		1.90	1.90	1.92
Control 2	1.72	1.72	1.65	1.72	1.70
Control 3	1.80	1.80	1.75	1.75	1.80
Control 4	1.70	1.80	1.77	1.68	1.68
Average	1.78	1.77	1.78	1.78	1.78
Average increase over viscosity of water	0.78	0.77	0.78	0.78	0.78
Experiment 1	1.76	1.61	1.59		1.72
Experiment 2	1.91	1.85	1.85		1.96
Experiment 3	1.74	1.72	1.63	1.55	1.66
Experiment 4	1.71	1.56	1.56		1.70
Experiment 5	1.80	1.73	1.69		1.78
Experiment 7	1.80	1.84	1.80	1.70	1.85
Experiment 9	1.75	1.70	1.65		1.79
Experiment 10	1.85	1.77	1.70		
Average	1.79	1.72	1.65	1.63	1.79
Average increase over viscosity of water	0.79	0.72	0.65	0.63	0.79
Per cent of initial increase	100	92	83	80	100

not satisfactory because of difficulty in controlling the "time-drop" described by du Noüy. Preliminary experiments indicated a probable increase in the surface tension following the administration of water, but because of the deviations in the method used the surface tension was not studied in detail.

Plasma volume (*table 6*).—The extent of the increase in the plasma volume varies in the different individual experiments, but in general the change is proportional to the degree of change in the hemoglobin con-

centration. In this series the increase on the average was from 695 to 790 cc., or 14 per cent. In these experiments only one blood sample was taken. They serve, therefore, as an additional control of the experiments in which the changes in the blood were followed in more detail. The plasma volumes observed in these experiments were approximately equal in magnitude to those found by Lee, Carrier and Whipple (1922). They gave dogs large amounts of water over a period of from three to three and a half hours, and found a decrease in the hematocrit reading and an increase in the plasma volume of 16 per cent on the average.

TABLE 6

Changes in the plasma volume and blood concentration following the administration of excessive amounts of water

EXPERIMENT	PLASMA VOLUME		HEMOGLOBIN		SERUM PROTEIN		SERUM VISCOSITY		PLASMA CHLORIDS	
	Normal value	After administration of water	Normal value	After administration of water	Normal value	After administration of water	Normal value	After administration of water	Normal value	After administration of water
	cc.	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*			*mgm.*	*mgm.*
10	620	670	100	84	7.3	6.7	1.92	1.70		
11	490	620	100	96						
12	460	490	100	89	6.1	4.9	1.65	1.50	620	411
13	750	860	100	95	6.5	5.8	1.83	1.74	621	451
14	720	1,010	100	96					680	455
15	820	920	100	87	7.0	6.2			651	411
16	1,015	1,000	100	99					606	430
17	650	730	100	89	7.6	5.8	1.76	1.63	620	475
18	860	905	100	95	6.0	5.5	1.70	1.59	620	441
19	570	610	100	91	8.1	7.2	1.95	1.83		
Average.....	695	790	100	92	6.9	6.0	1.80	1.66	633	440
Per cent of normal value.....	100	114	100	92	100	87	100	83	100	70

GENERAL DISCUSSION. The individual changes in the different constituents of the blood, although slight, are remarkably uniform in direction, character and degree. The changes noted become the more striking when one considers the usually observed constancy of composition of the blood and the failure of others uniformly to produce distinctive changes in man. This in part may be explained by the larger quantities of fluid that can be given to a dog. Lee, Carrier and Whipple observed similar changes in the dog, and since our preliminary report (Greene and Rown-

tree, 1924) these experiments have been confirmed by Chanutin, Smith and Mendel, and by Underhill and Sallick (1925).

No evidence of hemolysis or of increased destruction of erythrocytes, such as hemoglobinuria, was observed. The changes in the hemoglobin content cannot be explained on that basis. Furthermore, as will appear from the tables, the change in the hemoglobin content is accurately paralleled by that in the serum proteins.

The observed changes in the refractive index, viscosity, and surface tension of the serum, and of the specific gravity and total solids of the plasma, speak for a corresponding change in the percentage of the serum proteins. The albumin-globulin ratio in the serum, calculated from the refractive index and the relative viscosity by the method of Naegli (1919) is unchanged. This would indicate that the serum proteins are affected as a whole rather than that there is a disturbance in the proportion of the individual proteins.

The changes in the concentration of hemoglobin and of the serum proteins of necessity must be explained on the basis of a true dilution of the blood. The passage of an excess of the ingested water into the blood stream is indicated by the similarity in magnitude and the degree of parallelism in the changes in these two constituents. Furthermore, there is a direct increase in the plasma volume confirmatory of the other evidence for dilution.

The evidence of a true dilution of the blood in these experiments supports the view that hydremic plethora (oligocythemic hypervolemia) may be produced by the oral administration of excessive amounts of water. It is clear that the organism is able to compensate for physiologic quantities of water, but that when fluid is forced the compensating mechanism may fail and hydremia be produced.

II. ON THE NON-PROTEIN CONSTITUENTS OF THE BLOOD

In part I we reported the production of hydremic plethora in dogs by the oral administration of large amounts of water. In these animals there was an increase in the plasma volume with a corresponding reduction in the hemoglobin content of the blood and in the serum proteins.

The colloidal constituents of the blood, such as the serum proteins, are confined to the blood stream, and so respond passively to changes in the blood volume. The same is true of the hemoglobin, which is confined to the erythrocytes. The crystalloid constituents of the blood, on the other hand, may freely pass into and out of the blood stream, and need not necessarily respond in the same manner to the hydremia as do the colloids. While Haldane and Priestley did not find changes in the

TABLE 7

Changes in constituents of blood and urine following the administration of excessive amounts of water

Dog 183, May 11, 1923. Weight, 7.0 kgm.

TIME	WATER ADMINISTERED	TOTAL WATER	BLOOD		SERUM		URINE					REMARKS
			Hemoglobin	Urea	Chlorids, NaCl	Calcium	Volume	Urea	Urea, total	Chlorids, NaCl	Chlorids, total NaCl	
	cc.	*cc.*	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*cc.*	*per cent*	*gm.*	*per cent*	*gm.*	
Normal			100.0	36	681	12.1		1.104		0.65		
9:00	350											
10:00	350											
1 hour		700	85.5				5					
10:30	350											Vomited
11:00	350											
2 hours		1,400	90.6				180					
11:30	350											Vomited
12:00	350											
3 hours		2,100	89.7	30		12.5	165	0.185	0.650	0.01	0.035	
12:30	350											Vomited
1:00	350											
4 hours		2,800					185					
1:30	350											Salivated;
2:00	350											vomited
5 hours		3,500	89.0				260					
2:30	350											
3:00	350											
6 hours		4,200	82.6	18	551	10.9	240	0.074	0.510	0.00	0.000	
3:30	350											
4:00	350											
7 hours		4,900	82.0									
4:30	350											
5:00	350											
8 hours		5,600	81.4	16	541	10.1	225	0.056	0.238	0.00	0.000	
24 hours			93.6	11	631	12.7	1,040	0.063	0.656	0.02	0.208	Recovered

hemoglobin content of the blood in man after taking large quantities of water, Priestley (1915–1916) was able to show a slight decrease in the electrical conductivity of the serum in these same experiments. Christie and Stewart (1922), however, found but little change in the serum of patients with diabetes insipidus during periods of thirst or of high water intake.

EXPERIMENTAL PROCEDURES. The administration of water and the taking of blood samples was the same as in the previous report. The

TABLE 8

Changes in constituents of blood and urine following the administration of excessive amounts of water

Dog 187, June 17, 1923

TIME	WATER ADMINISTERED	TOTAL WATER	WEIGHT	BLOOD		SERUM			URINE					REMARKS
				Hemoglobin	Urea	Chlorids, NaCl	Sodium	Carbon dioxid capacity	Volume	Urea	Urea, total	Chlorids, NaCl	Chlorids, NaCl, total	
	cc.	*cc.*	*kgm.*	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*volume per cent*		*per cent*	*gm.*	*per cent*	*gm.*	
Normal			10.0	100.0	25	686	349	38	50	0.731	2.56	0.130	0.455	
9:00	500													
9:30	500													
10:00	500													
10:30	500													Vomited
11:00	500													Vomited
11:30	500													Salivated
3 hours		3,000		95.0	22	511	350	26	24					
12:00	500													
12:30	500													Vomited
1:00	500													Salivated
3 hours		4,500	10.6	90.5	23	506	320	24	17	1.290	0.53	0.256	0.108	Convulsion
24 hours			9.7	91.5	29	656	331	39	1,000	0.550	5.50	0.066	0.660	Recovered

specific conductivity of the blood serum at 25°C. was determined by the use of a modified Ostwald cell and a ten-meter bridge. The cell constant was carefully checked against a standard solution of potassium chlorid before and after each series of determinations. The cell was especially constructed to permit readings on 1 cc. samples of serum. The mean of three concordant determinations, each made on a fresh portion of serum, was used in the calculation of the conductivity.

Freezing points were determined in the usual manner. The plasma chlorids were determined by titrating the tungstic-acid filtrate according

to the method of Smith. The inorganic cations were determined by the methods of Kramer and Tisdall. The blood urea was determined by the urease method, and the blood sugar by the method of Folin and Wu.

TABLE 9

Changes in constituents of blood following the administration of excessive amounts of water

Dog 186, June 13, 1923. Weight of dog, 14.3 kgm.

TIME	WATER ADMINISTERED	TOTAL WATER	BLOOD		SERUM				REMARKS
			Hemoglobin	Urea	Chlorids, NaCl for each 100 cc.	Sodium for each 100 cc.	Calcium for each 100 cc.	Potassium for each 100 cc.	
	cc.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Normal			100.0	29.2	621	422	11.5	21.7	
9:30	700								
10:00	700								Vomited
10:30	700								
11:00	700								Vomited
11:30	700								
12:00	700								
3 hours		1,400	97.5	20.3	502	308		19.2	
12:30	700								Watery stool
1:00	700								Vomited
1:30	700								
2:00	700								
2:30	700								
3:00	700								Twitched
6 hours		8,400	95.2	20.3	481	249	10.5	14.0	Vomited
3:30	700								
4:00	700								Vomited
4:30	700								
5:00	700								Twitched
8 hours		11,200	90.0	22.5	456	237	9.4	19.8	Vomited
24 hours			102.3	35.2	571			22.8	Recovered

EXPERIMENTAL DATA. Protocols of typical experiments are shown in tables 1 and 2. The changes in the different non-protein constituents are shown in more detail in tables 3 to 15.

The electrical conductivity of the serum decreased progressively during the course of the experiments (table 10). This decrease on the average

was from 108 to 92 with a return to 102 at the twenty-four-hour reading. This corresponds on the average with a decrease of 15 per cent in the conductivity of the serum.

The samples of blood drawn at each time were small, and too little serum was obtained to permit of the satisfactory determination of the freezing point in all specimens. The protocols of two satisfactory experiments (tables 1 and 2), however, show that there was an elevation of the freezing point during the forced administration of water. The decrease in the molecular concentration of the serum indicated by this change in the freezing point is in entire accord with the changes in the conductivity.

The plasma chlorids, calculated as milligrams of sodium chlorid for

TABLE 10

Changes in the specific conductivity of the serum following the administration of excessive amounts of water

EXPERIMENT	NORMAL VALUES	TIME IN HOURS			
		3	6	8	24
		1×10^{-4} 25°C.			
1	114	95	93		108
2	107	97	93		104
3	110	104	93	95	106
4	106	91	84		103
5	107	99	98		93
6	104		89		104
7	111			90	99
Average	108	97	92	92	102
Per cent of normal value	100	90	84	85	95

each 100 cubic centimeters, on the average decreased from 639 to 500 during the first three hours of the experiment, and to 484 at the conclusion (table 11). Recovery was not complete within the time limit studied, for they only returned to 576 in the twenty-four-hour sample.

The concentration of sodium in the serum in the average of four experiments decreased from 381 mgm. per cent to 277 mgm., a change of 27 per cent (table 12). This corresponds closely to the changes in the chlorids. The potassium content of the serum in one experiment decreased from 24 to 18 mgm., or by 25 per cent (table 9). The changes in the calcium (table 12) were not so marked. The average decrease in four experiments was from 11.4 to 9.6 mgm., or 16 per cent.

The loss in alkali was also shown in some experiments by a decrease

in the alkali reserve of the serum (table 13). The changes in the carbon dioxid capacity of the plasma were quite variable. Many of the animals developed rapid shallow respiration, and the varying effects of overventilation, or other respiratory disturbance or of the convulsions themselves, will serve to explain the observed irregularities in the alkali reserve.

The organic crystalloids in the serum did not show the regularity in behavior that characterizes the inorganic salts. The blood urea on the

TABLE 11

Changes in the plasma chlorids following the administration of excessive amounts of water

	NORMAL VALUES	TIME IN HOURS			
		3	6	8	24
		Milligrams per cent			
Control 1	663	681	655	683	700
Control 2	647	655	650	644	710
Control 3	664	654	628	645	652
Control 4	642	663	658	642	628
Average	654	662	648	653	672
Per cent of normal value	100	101	99	100	103
Experiment 8	631	476	476		586
Experiment 9	636	551	521	536	496
Experiment 10	681		551	541	631
Experiment 11	621	502	481	456	571
Experiment 12	686	511	506		656
Experiment 13	660	515	450		525
Experiment 14	610	500	450	440	
Experiment 15	690	504	449		
Experiment 16	557		562	448	
Experiment 17	618	445			
Average	639	500	494	484	576
Per cent of normal value	100	78	77	76	90

average decreased from 28 to 19 mgm., or 32 per cent (table 14). This is the most marked change observed in any of the blood constituents. The changes in the blood urea varied widely in the different experiments, depending largely on the degree of diuresis produced in the individual experiments. When diuresis fails, as in some experiments, the blood urea may even be increased.

The blood sugar was approximately constant during the early portion of the experiment (table 15). At the time of the appearance of convulsions,

however, the blood sugar increased markedly though the degree of hyperglycemia produced was never extreme.

TABLE 12

Changes in the sodium and calcium in the serum following the administration of excessive amounts of water

EXPERIMENT	NORMAL VALUES	TIME IN HOURS			
		3	6	8	24
		Milligrams per cent			
Sodium					
11	422	308	249	237	
12	342	350	320		321
18	384	281	289		338
19	378	285	252		330
Average....	381	306	277		330
Calcium					
10	12.1	12.5	10.9	10.1	12.7
11	11.5		10.5	9.4	
18	10.3	9.6	9.0	9.3	9.5
19	11.4	10.4	10.3		10.5
Average....	11.4	10.8	10.2	9.6	10.9

TABLE 13

Changes in the carbon dioxid capacity of the plasma following the administration of excessive amounts of water

EXPERIMENT	NORMAL VALUES	TIME IN HOURS			
		3	6	8	24
		Per cent by volume			
20	47	52	38		
21	48	30	54		
15	46	38	36		
16	52	39	38	34	
22	42		39		
17	44	24			
18	43	43	55	44	45
19	48	45	54		49
Average....	46	39	52		

GENERAL DISCUSSION. In any interpretation of the effects of hydremic plethora (oligocythemic hypervolemia) on the volume and composition

of the blood, distinction must be made between the effect of the dilution alone and the response to that dilution. The total amounts of

TABLE 14

Changes in the blood urea following the administration of excessive amounts of water

EXPERIMENT	NORMAL VALUES	TIME IN HOURS			
		3	6	8	24
		Milligrams per cent			
10	36	30	18	16	11
11	29	20	20	22	35
12	25	22	23		29
13	35	31	36		40
14	14	22	16		
17	27	35			
15	12	24	47		
16	19	18	27	23	
20	25	22	35	28	
21	17	48	24		
22	15	26	40		
23	24	28	20		
Average.....	23	27	28	22	29
Per cent of normal value..	100	112	122	96	126

TABLE 15

Changes in the blood sugar following the administration of excessive amounts of water

EXPERIMENT	NORMAL VALUES	TIME IN HOURS			
		3	6	8	24
		Milligrams per cent			
24	95	81	107	130	81
13	85	94	187		88
14	85	84	140		
15	97	94	91		
16	108	93			
18	87	108	121	193	98
19	97	140	222		93
Average.....	93	99	144		90
Per cent of normal value..	100	106	155		97

circulating hemoglobin and of serum proteins apparently are not affected by the increase in the plasma volume. Changes in the concentration

of these substances, as shown in part I, afford a convenient index to the degree of hydremia produced.

The molecular and electrolytic concentration of the serum, as shown by the specific conductivity and the depression of the freezing point, decreases following the administration of water. The changes, however, are similar in degree to those observed in the hemoglobin, and the differences are not sufficiently marked to indicate the influence of factors other than dilution alone.

The salt content of the serum, however, shows striking changes. The chlorid content decreases on the average by as much as 25 per cent. This decrease is nearly double that in the hemoglobin, a variation that cannot be attributed to the effects of dilution alone. Neither can this be explained on the basis of sodium chlorid being washed from the body. Chlorids are among the threshold bodies and are not excreted in the urine when the concentration in the blood plasma falls below this threshold level. Some of the dogs in these experiments developed marked diuresis (table 7), but the urine rapidly became chlorid-free. Other animals developed nearly complete anuria (table 8), even though the water was forced according to the routine procedure. In neither instance was there a sufficient urinary output of chlorid to account for the changes in the blood. A trace of chlorids is present in the fluid vomited by these animals, but this again is inconsiderable by comparison with the changes in the body as a whole. The relation of the tissues to the changes in the blood will be reported on later.

Underhill and Sallick concur with the view of Rowntree that the development of water intoxication is associated with a disturbance in the salt-water equilibrium in the body. Metabolism experiments covering twenty-four-hour periods show in general a marked increase in the excretion of chlorids accompanying the administration of water. That at first might seem to explain the whole problem. The study of shorter intervals, however, shows that the greater part of the diuresis, and so the excretion of salt, may occur during the period of recovery, after the development of toxic symptoms and the cessation of the water administration. As already outlined, the urinary excretion, especially of chlorids, may be minimal at the time of the development of symptoms.

The sodium and potassium content of the serum decreased in a fashion similar to the changes manifested by the chlorids, although the excretion was studied less completely than was that of chlorids. The decrease in the alkali reserve of the blood may well be due in part to this loss of base.

The calcium content of the serum does not decrease as markedly as the other inorganic constituents studied. The magnitude of the change in the calcium is much less than that in the chlorids, and more nearly corresponds to the degree of change in the hemoglobin and serum proteins.

The calcium in the serum is partially combined with the protein, and only a relatively small proportion is present in the ionized form. This perhaps will explain the quantitative difference in the behavior of the different salts.

Different animals show considerable variation in the behavior of the urea. When there was no diuresis or actual anuria during the experiment the blood urea did not change, or even increased slightly. In those experiments in which diuresis was marked, the urea content of the blood was the most decidedly reduced. The concentration of urea in the urine decreased markedly during the diuresis, but not enough to prevent the washing out of an appreciable quantity of urea by the excess of fluid.

The blood sugar differed from the other constituents of the blood in remaining at a constant level, or even increasing slightly, with the development of hydremia. The mechanism normally regulating the blood sugar level is not affected by the administration of water and the dilution is amply compensated.

CONCLUSION

From these experiments it seems evident that in dogs the forced administration of excessive amounts of water produces demonstrable changes in the volume and composition of the blood. This dilution is shown by a reduction in the hemoglobin and serum proteins as well as by an increase in the plasma volume. The electrolytes in the serum are likewise reduced but the concentration of chlorides and sodium decreases to a greater extent than would be expected from the degree of dilution. The behavior of the non-electrolytes, urea and sugar is apparently determined by factors other than the dilution and so is partially independent of the water administered.

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THE EFFECT OF THE ADMINISTRATION OF EXCESSIVE AMOUNTS OF WATER ON BODY TEMPERATURE

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Received for publication October 25, 1926

The body temperature is one of the physiologic constants of the organism. Heat is liberated by the oxidation of the different metabolites, the rate of its production depending on various factors such as the available foodstuffs and their varying dynamic action, the effect of endocrine products and the energy demands of the body. The distribution of this heat through the body and its final dissipation is made possible largely through the thermal properties of water, which constitutes such a large proportion (more than 75 per cent) of the body weight.

The water content of the body likewise is a constant, for although water normally is ingested in amounts greater than required by the body, the excess is excreted with great rapidity and the water balance thus maintained at the level of maximal physiologic efficiency. Numerous observers have reported the effects of deprivation of water but it is only recently that Rowntree (1923) has pointed out the possibility, as in these experiments, of forcing water ingestion to such a degree that physiologic disturbances result.

Henderson (1913) has emphasized the importance of the characteristic physical and chemical properties of water for the maintenance of life. Distribution and dissipation of heat throughout the body is made posssible by reason of the mobility and ideal thermal properties of water, that is, the high specific heat which favors storage, the high thermal conductivity which provides for a rapid equalization of temperature within the tissues of the body and the extreme heat of vaporization which favors the rapid elimination of heat (Barbour, 1921).

The regulation of the dissipation of heat is under the control of the nervous system acting through 1, the vasomotor mechanism which determines the distribution of blood throughout the tissues and particularly the peripheral flow; 2, the sudatory mechanism which controls the secretion of sweat, which in man is of especial importance in temperature regulation, and 3, the respiratory mechanism for the evaporation of water through the lungs. The insensible perspiration, which is important in animals

such as the dog. The nervous control of these different mechanisms, acting either peripherally or on the medullary centers, is responsible for the homothermic condition of mammals, and destruction of the nervous control, as by decerebration, causes its conversion to a poikilothermic state.

The thermal properties of water have been employed in medicine since ancient times. In discussing the importance of hydrotherapy in the treatment of typhoid fever, Osler says, "The use of water, inside and outside, was no new treatment in fevers at the end of the eighteenth century, when James Currie (a friend of Burns and the editor of his poems) wrote his *Medical Reports on the Effects of Water, Cold and Warm, as a Remedy in Fevers and other Diseases.* In this country it was used with great effect and recommended by Nathan Smith, of Yale. Since 1861, the value of bathing in fevers has been specially emphasized by the late Dr. Brand, of Stetten." In our own time we have witnessed the general adoption of the "water treatment" of typhoid fever, inaugurated by Brand. The importance of hydrotherapy is now recognized, and also the ingestion of water is forced almost as a routine in febrile conditions. The emphasis with the latter, however, has largely been placed on the elimination of toxins by sweating or an increased excretion of urine.

Quite another phase of the relation of water to body temperature has attracted attention in recent years. Crandall, in 1899, described febrile attacks, occurring in infants during inanition, which he showed to be due to thirst, for they disappeared on the administration of water. Finkelstein, in 1908, described fever in children following the administration of hypertonic solution of lactose, sodium chlorid or glucose, a condition since referred to as salt fever or sugar fever.

Balcar, Sansum and Woodyatt (1919) produced glucose fever in adult human beings and in dogs through the intravenous administration of concentrated solutions. They produced fever in animals with a temperature of 125.6°F. after the injection of glucose and 111.0°F. after the injection of sodium chlorid and lactose. They ascribed the fever to deprivation of water with consequent disturbance in the equilibrium between the "free" and "bound" water in the blood and tissues. Keith and Whelan (1926) on the other hand, found that, when care is taken to exclude all possible extraneous toxic materials in the preparation of the solutions for injection, marked and rapid loss of water can be produced in dogs without marked hyperpyrexia.

Seibert (1924–25) has made a study of fever producing samples of distilled water and has demonstrated the presence of a "pyrogen" arising from bacterial contamination. Andrews (1926) has also studied the salt fevers in animals and emphasized the fact that in experiments on dogs care must be taken to avoid interference with the respiratory exchange. He was unable to produce marked degrees of fever in animals when

the respiration and evaporation from the tongue and mouth were unimpeded. Binding the jaws, however, prevented buccal loss of water. Such interference with the heat regulating mechanism regularly led to the appearance of fever.

METHODS OF EXPERIMENTATION. The methods employed in the experiments were those previously described by Rowntree. Water in quantities of 5 per cent of the body weight was given to dogs by a stomach tube at half-hourly intervals. The temperature of the water in the different experiments varied from 0 to 50°C. The room temperature was between 19° and 24°C. The animals were tied down in as comfortable a position as possible and the temperature determined by placing the metal bulb of a Tagliabue continuously registering thermometer in the rectum approximately 6 cm. above the anal sphincter. The temperature was thus registered throughout the course of the experiment. The administration of water was continued to the approximate limit of tolerance as shown by the onset of convulsions.

Rowntree has pointed out that the appearance of convulsions and the symptoms of "water intoxication" were independent of the temperature at which the water was given. In this earlier report temperature readings were noted in three animals without significant changes being observed. It was only after the adoption of the sensitive recording thermometer that the magnitude and significance of the temperature changes appeared. The tying down and consequent limitation of muscular activity may also be a factor in determining the changes observed.

CONTROL EXPERIMENTS. Four control experiments were carried out with the animals lying quietly in a room at a temperature between 22° and 25°C. A characteristic result is shown in figure 1, chart 1. The initial temperature was 37.9°C. During the course of the first hour it rose to 38.3°C. and was sustained at that level for four hours, with a maximal variation throughout of 0.1°C. This animal, when given water at a temperature of 39.0°C., according to the method of administration described, showed a drop in temperature of 1.0°C. in less than four hours. Similar control experiments were made by Keith and Whelan. In one of our control experiments (fig. 1, chart 2) the initial temperature was 37.9°C. This dropped within three hours to 37.1°, a decrease of 0.8°C. During an actual experiment, when water was given at 39°C. the temperature dropped from 37.7° to 34.9°, a decrease of 2.8°C. in the course of six hours.

EXPERIMENTAL DATA. The temperature was studied in twenty-eight dogs. The results in fifteen of these appear in the accompanying table. The circular instrument chart is graduated in degrees Fahrenheit and must be read in a clockwise manner. The data in the figure have been transferred to a linear chart graduated in degrees for greater ease in reading.

Response to water at 0°C. Three experiments were carried out with water at 0°C. The temperature curves were essentially the same in all, that in Figure 1, Chart 3 serving as a basis of discussion. The initial temperature of the animal was 39.0°, the lowest 35.4° and the final 36.4°C. The decrease in temperature immediately after each dose of water is adequately shown, as is the attempt of the organism to reëstablish the normal temperature level. The convulsions in this particular instance coincide with the point of maximal change in temperature, 3.6°C., but this is not necessarily so. Convulsions per se have no immediate effect on the temperature which rises gradually and continuously after the administration of water is stopped.

Response to water at 39°C. Four experiments were carried out with the water at 39°C., the maximal decrease in each being 2.9°, 2.3°, 1.7° and 3.7°

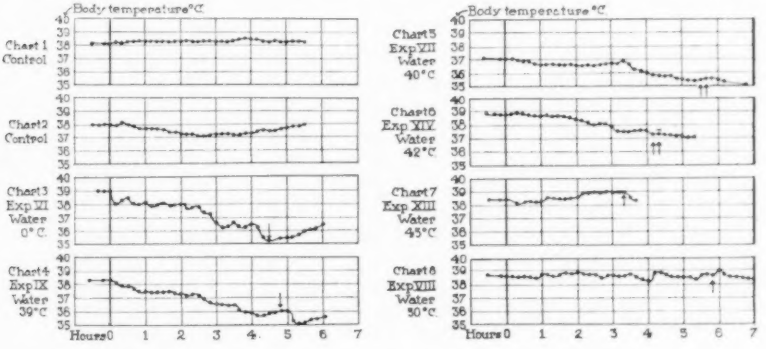
Body temperature °C
Chart 1 Control
Chart2 Control
Chart3 Exp XI Water 0°C
Chart4 Exp IX Water 39°C
Chart5 Exp XII Water 40°C
Chart6 Exp XIV Water 42°C
Chart7 Exp XIII Water 45°C
Chart8 Exp VIII Water 50°C
Hours 0 1 2 3 4 5 6 7

Fig. 1. Temperative curves of controls and to show the effect of the administration of water at various temperatures. The arrows indicate the onset of convulsions.

C. respectively. Chart 4, figure 1 will serve as an example, the initial temperature being 38.3° and the final temperature 35.4°. It will be seen that with water at 39.0°C. the body temperature falls continuously, although slight irregularities appear following each successive administration of water. Convulsions appeared at the beginning of the sixth hour during a period of slight, relatively increased temperature, and were followed within the next twenty minutes by a drop of 1.1°C. with a subsequent rise when the administration of water was stopped.

Response to water at 40° to 42°C. The results of two experiments are shown in figure 1, charts 5 and 6, with a maximal decrease of 2.4° and 1.6° respectively despite the fact that the temperature of the water administered was slightly above that of the body. The onset of convulsions had no recognizable influence on the temperature curve.

Response to water at 45°C. The response in a single experiment to water at 45°C. is shown in figure 1, chart 7. The maximal decrease in temperature was 0.3°C. while an increase of more than 0.6°C. was recorded at one time. The administration of each dose of water caused a slight rise in body temperature, with a slow return to the normal. Convulsions had no effect on the curve. It would appear, therefore, that water at 45°C. will sustain the body temperature at an approximately normal level even though given in excess.

Response to water at 50°C. Water at 50° C. is as hot as the animal can bear in large amounts without undue discomfort; consequently water at

Summary of results

EXPERIMENT	WEIGHT		TEMPERATURE OF WATER, °C.	BODY TEMPERATURE AT THE END OF EACH HOUR, °C.									MAXIMAL DROP IN TEMPERATURE °C.	DURATION OF EXPERIMENT		RESULT
	Before	After		At start	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours	8 hours		Hours	Minutes	
	kgm.	*kgm.*														
6	11.9	13.5	0	39.0	38.2	37.9	36.6	36.3	35.4	36.4			3.6	4	30	Recovered
4	10.75	11.8	0	38.3	37.7	37.4	36.9	34.9	36.2	36.9	37.0	37.5	3.4	5		Recovered
5	16.3	17.9	0	38.1	36.8	36.6	37.1	36.8					1.5	4	45	Recovered
3	5.82	6.8	28	38.3	37.8	37.7	37.8	37.7	37.6	37.4	36.9	37.4	1.4	5	39	Died
11	8.54	9.2	38	38.4	37.7	38.0	37.8	36.8	36.7				1.7	3	20	Recovered
9	10.1	11.3	39	38.3	37.5	37.3	36.7	35.8	36.0	35.4			2.9	5		Recovered
10	9.55	11.4	39	38.3	38.1	37.7	37.0	37.1	37.2	37.4	36.4	36.0	2.3	7	35	Recovered
1			39	38.5	38.0	37.3	37.6	36.8					1.7	3	25	Died
15	6.2	6.9	39	38.1	37.3	36.7	36.2	34.4					3.7	3	50	Died
12	6.9	8.3	40	37.0	36.6	36.6	36.6	36.0	35.4	35.4			2.4	5	42	Died
14	8.25	9.34	42	38.9	38.7	37.9	37.9	37.6	37.3				1.6	4	10	Recovered
13	2.56	9.05	45	38.4	38.1	38.4	39.0	38.3					0.3	3	47	Recovered
8	11.5		50	38.6	38.8	38.8	38.6	38.9	38.6	38.8	38.2		0.0	5	40	Recovered
2			50	38.0	38.4	38.3	38.2	38.1	37.8	37.8	37.7	37.8	0.3	8	27	Recovered
7	15.95		50	37.7	37.4	37.4	37.5	38.0	37.3	37.2			0.5	4	48	Recovered

temperatures greater than this has not been employed. Three experiments were carried out. Vomiting was marked and consequently the convulsions were delayed in onset. The temperature remained fairly constant, the greatest drop in the three animals being 0.5°C. Figure 1, chart 8 indicates the course of events. At first a slight tendency to increase in temperature is apparent with a return to normal with each subsequent injection. For instance in the beginning of the fourth hour, the administration of water was promptly followed by an increase of 0.3°C. then by a gradual fall in temperature until it was raised to a similar extent by water given again at the end of the next half-hour. Convulsions de-

veloped at the beginning of the seventh hour. The final temperature was approximately 0.2° lower than the initial reading.

Discussion. From these experiments it seems evident that the forced ingestion of excessive amounts of water in dogs may result in a definite decrease in the body temperature even though the water is administered at a temperature equal to or even slightly above that of the body. The condition of the animals and the development of convulsions preclude accurate metabolism studies. There is evidence of increased intracranial pressure but no evident increase in the pulmonary ventilation. The animals are usually depressed or comatose, and cold to the touch. It would seem likely, therefore, that the fall in temperature results from a decrease in the heat production rather than an increase in the dissipation of heat. However, the possible effect of full vessels with increased peripheral circulation is not entirely excluded.

Our previous studies indicate that the forced administration of water is associated with an increase in the blood volume and dilution of the blood with a further reduction in the inorganic salts. Further studies will be necessary to show the relations between such changes and the conceptions of the physiologic importance of "free" and "bound" water in the organism.

CONCLUSION

A decrease in the body temperature is produced in dogs by the forced administration of excessive amounts of water even though the water is given at a temperature equal to or slightly above that of the body.

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